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Effects of community composition and global change on the functioning of experimental marine phytoplankton communities

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The only real voyage of discovery consists not in seeking new landscapes, but in having new eyes.

— Marcel Proust

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Summary

Humans are altering the composition of biological communities through a variety of activities at all scales, from local to global. These changes in components of the Earth's biodiversity cause concern for ethical and aesthetic reasons, but they also have a strong potential to alter ecosystem properties and the goods and services they provide to humanity. Since the industrial revolution, atmospheric carbon dioxide (CO_2) increased from 280 to 380 µatm and is expected to further increase to 700 µatm by the year 2100. Ocean acidification is the consequence of increasing atmospheric CO_2, which dissolves in seawater and subsequently increases seawater acidity and decreases carbonate ion concentration. Changes in carbonate chemistry can act both as fertilizer in case CO_2 is a limiting resource and as stressor, particularly for calcifying organisms. Ocean acidification represents a pervasive environmental change that is predicted to affect a wide range of species, yet our understanding of the emergent ecosystem impacts is very limited.

Two most challenging questions largely remain uncertain. Firstly, how much of the expected change in community functioning due to elevated CO_2 is owing to either changes in the physiology of individual species or in the relative abundance of species or is there a hint towards evolutionary adaptation? Secondly, how do effects of community composition on ecosystem functioning compare to direct effects of ocean acidification?

In chapter I, I tested whether varying initial dominance scenarios lead to different competitive outcomes and subsequently translate into altered community functioning. I used experimental communities consisting of four naturally co-occuring coccolithophore species and manipulated initial community structure by creating five different dominance scenarios: (1) all species contributing evenly to initial biomass, and (2-5) one of each species contributing 4x that of the remaining three species to total initial biomass. I was able to show that priority effects in the communities caused the initially dominant species to remain dominant during the stationary phase in three out of four cases. However, despite varying carrying capacities when species were grown in monocultures and different dominant species, community functioning was unaffected. I suggest that selective and facilitative effects were responsible for the equalization of community functioning.

In chapter II, I used three of the four coccolithophore species used in chapter I and explored the effect of initial community composition in combination with ocean acidification on community biomass. In particular, I tackled the question of how much of the expected change in community functioning due to elevated CO_2 is owing to either direct changes in the physiology of species or indirect ecological changes in the relative abundance of species. In order to complete the picture, I additionally indirectly tested for evolutionary adaptation to elevated CO_2. Contrary to my expectation I found neither a significant physiological effect nor

an ecological effect of elevated CO_2 on biomass at bloom peak. I concluded that the lacking effect on ecosystem functioning in this particular model system in response to elevated CO_2 was likely caused by community reorganization due to evolutionary adaptation.

In chapter I and II, community functioning at bloom peak was affected neither by initial community composition nor ocean acidification. The communities in both studies however, consisted only of coccolithophores. In order to overcome this limitation, in chapter III, I used communities harboring a variety of functional groups and tested the hypothesis that initial community composition and elevated CO_2 are equally important to the regulation of phytoplankton biomass. I was able to show that initial community composition had a significantly greater impact than elevated CO_2 on phytoplankton biomass, which varied largely among communities. Furthermore, I showed that depending on initial community composition, elevated CO_2 selected for larger sized diatoms, which led to increased total phytoplankton biomass.

Overall, the results suggest that when looking at more than one functional group, initial community composition can have a much greater effect on biomass than elevated CO_2. Consequently, the importance of ocean acidification hitherto appears to be overestimated whereas the effect of community composition has been largely overlooked, although it is among the dominant drivers of changes in ecosystem functioning. Because phytoplankton functioning depends on trait composition, it remains a major challenge to understand how phytoplankton communities will reorganize in response to climate change in order to predict the impact on future oceans' ecosystems. Inherently, using independent natural communities, instead of directly manipulating biodiversity, limits the possibility for mechanistic explanation. For future research I suggest to overcome this problem by using one known source-community in which biodiversity (i.e. the loss or distribution of given traits) is manipulated in a non-random approach.

Zusammenfassung

Der Mensch beeinflusst die Zusammensetzung biologischer Gemeinschaften durch eine Vielzahl von Aktivitäten auf lokaler sowie auf globaler Ebene. Die Auswirkungen veränderter Biodiversität geben Ursache für ethische und ästhetische Bedenken; allerdings haben sie auch großes Potential, die Eigenschaften von Ökosystemen und somit die Leistungen, die diese dem Menschen erbringen, zu beeinflussen. Seit Beginn der industriellen Revolution ist die Konzentration des atmosphärischen Kohlendioxids (CO_2) von 280 auf 380 µatm angestiegen und es wird erwartet, dass sie bis zum Jahr 2100 weiter auf 700 µatm ansteigt. Eine Folge des ansteigenden atmosphärischen CO_2-Gehalts ist die Ozeanversauerung. Steigt in der Atmosphäre der CO_2-Gehalt, nimmt die Konzentration des Gases auch in den oberflächennahen Schichten der Ozeane entsprechend zu. Die Reaktion des Kohlendioxids im Meerwasser führt zu einer Abnahme des pH-Wertes und der Karbonationenkonzentration. Veränderungen in der Karbonatchemie des Meerwassers können sowohl als Dünger, im Fall, dass CO_2 eine limitierende Ressource ist, oder als Stressor, besonders für kalzifizierende Organismen, wirken. Ozeanversauerung stellt eine tiefgreifende Veränderung der Umwelt dar, die voraussichtlich viele Arten beeinflussen wird; dennoch ist unser Verständnis für die daraus hervorgehenden Auswirkungen auf das Ökosystem sehr begrenzt.

Zwei herausfordernde Fragen bleiben größtenteils unbeantwortet. Erstens, wieviel der durch erhöhten CO_2-Gehalt zu erwartenden Veränderung in der Ökosystemfunktion entsteht aufgrund von entweder Veränderungen in der Physiologie einzelner Arten oder der relativen Häufigkeit von Arten oder gibt es einen Hinweis auf evolutive Anpassung? Zweitens, wie stehen Effekte von Gemeinschaftszusammensetzung auf die Ökosystemfunktion da im Vergleich zu direkten Effekten von Ozeanversauerung?

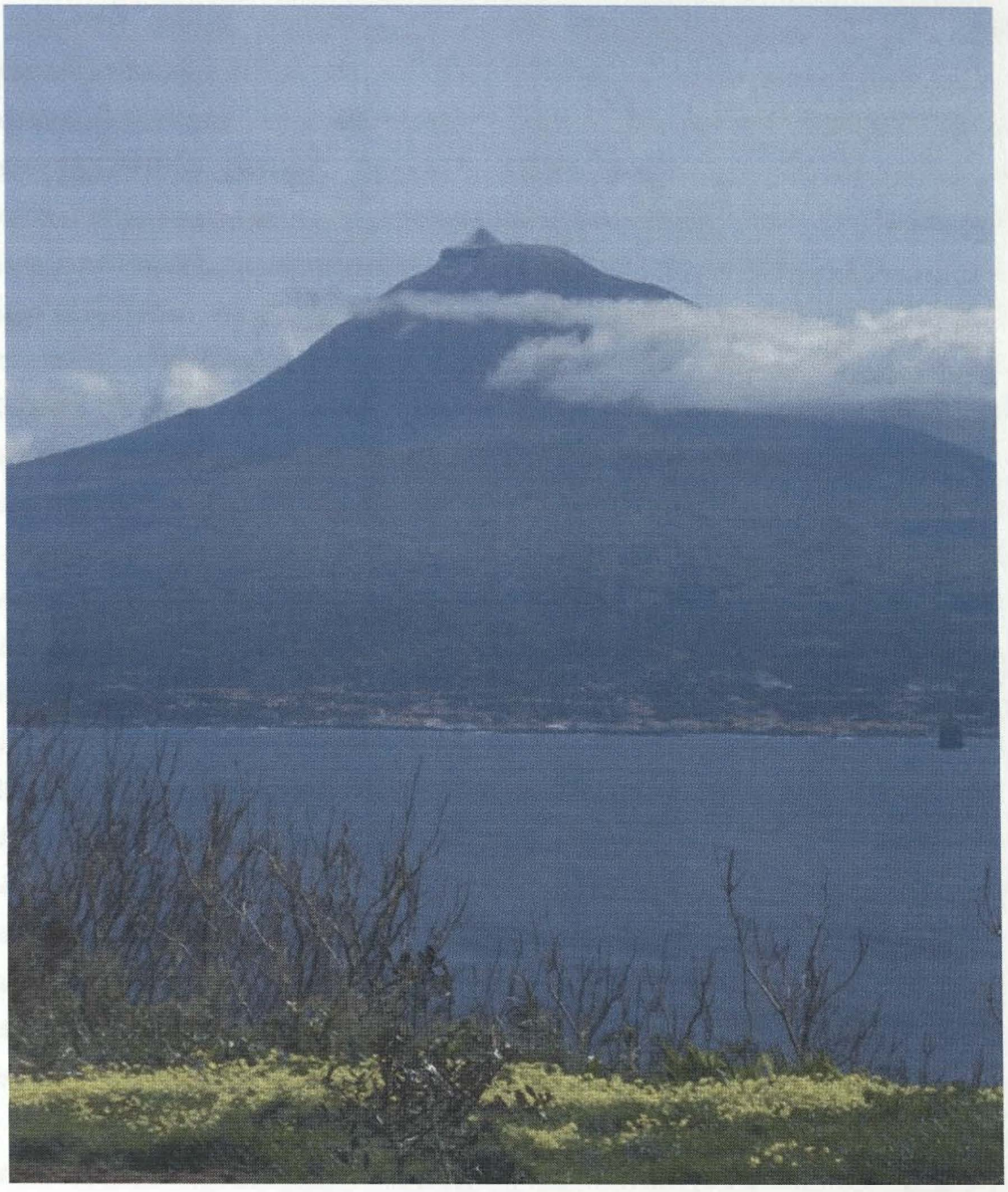
In Kapitel I, habe ich untersucht, ob differierende initiale Dominanzszenarien zu unterschiedlichen Ergebnissen führen und sich diese folglich in veränderter Ökosystemfunktion wiederspiegeln. Dazu habe ich experimentelle Gemeinschaften, bestehend aus vier natürlich gemeinsam vorkommenden Arten von Coccolithophoriden, verwendet und die initiale Gemeinschaftsstruktur manipuliert, indem ich fünf verschiedene Dominanzszenarien kreiert habe: (1) alle Arten tragen gleichmäßig zur initialen Biomasse bei, und (2-5) jede Art trägt einmal viermal mehr als die übrigen drei Arten zur initialen Biomasse bei. Ich konnte zeigen, dass Prioritätseffekte in den Gemeinschaften dazu geführt haben, dass die initial dominante Art in drei von vier Fällen auch in der stationären Phase dominant geblieben ist. Jedoch trotz unterschiedlicher Tragekapazitäten, wenn die Arten in Monokultur gewachsen sind, und differierender dominanter Arten, gab es keinen Effekt auf die Ökosystemfunktion. Die Ergebnisse deuten darauf hin, dass selektive und fördernde Effekte für den Ausgleich der Ökosystemfunktion waren.

In Kapitel II, habe ich drei der bereits in Kapitel I verwendeten Arten benutzt und den Effekt von initialer Gemeinschaftszusammensetzung im Zusammenspiel mit Ozeanversauerung auf Gemeinschaftsbiomasse untersucht. Insbesondere bin ich die Frage angegangen, wieviel der zu erwartenden Veränderung in der Ökosystemfunktion durch erhöhten CO_2-Gehalt durch entweder direkte Veränderungen in der Physiologie der Arten oder indirekte ökologische Veränderungen in der relativen Häufigkeit der Arten verursacht wird. Um das Bild zu vervollständigen, habe ich zusätzlich indirekt für evolutive Anpassung an einen erhöhten CO_2-Gehalt getestet. Entgegen meiner Erwartung, gab es weder einen signifikanten physiologischen noch ökologischen Effekt von erhöhtem CO_2-Gehalt auf die Biomasse zum Höhepunkt der Algenblüte. Ich habe gefolgert, dass der fehlende Effekt von erhöhtem CO_2-Gehalt auf die Ökosystemfunktion in diesem speziellen Modellsystem sehr wahrscheinlich auf evolutive Anpassung zurückzuführen ist.

In Kapitel I und II, wurde die Ökosystemfunktion weder durch initiale Gemeinschaftszusammensetzung noch durch Ozeanversauerung beeinflusst. Die Gemeinschaften in beiden Studien bestanden jedoch lediglich aus Coccolithophoriden. Um diese Einschränkung zu überwinden, habe ich in Kapitel III Gemeinschaften verwendet, die eine Mehrzahl funktioneller Gruppen beherbergen, und die Hypothese getestet, dass initiale Gemeinschaftszusammensetzung und erhöhter CO_2-Gehalt gleichermaßen wichtig für die Regulierung der Phytoplanktonbiomasse sind. Ich konnte zeigen, dass initiale Gemeinschaftszusammensetzung einen signifikant größeren Effekt als erhöhter CO_2-Gehalt auf die Phytoplanktonbiomasse hat, welche sich in hohem Maße zwischen den Gemeinschaften unterschied. Des Weiteren konnte ich zeigen, dass, abhängig von initialer Gemeinschaftszusammensetzung, erhöhter CO_2-Gehalt für größere Diatomeen selektiert hat, was letztendlich zu erhöhter totaler Phytoplanktonbiomasse geführt hat.

Insgesamt weisen die Ergebnisse darauf hin, dass, sobald mehr als eine funktionelle Gruppe betrachtet wird, initiale Gemeinschaftszusammensetzung sehr viel größere Auswirkungen auf die Biomasse heban kann als ein erhöhter CO_2-Gehalt. Folglich erscheint es, dass bisher die Wichtigkeit von Ozeanversauerung im Vergleich zu initialer Gemeinschaftszusammensetzung überschätzt wurde, obwohl letztere zu den wichtigsten Treibern von Ökosystemfunktion zählt. Da das Funktionieren von Phytoplanktongemeinschaften stark von der Zusammensetzung funktioneller Eigenschaften abhängt, bleibt es eine große Herausforderung zu verstehen, wie sich Phytoplanktongemeinschaften in Folge des Klimawandels neuordnen werden, um die Auswirkungen für das künftige Ökosystem Ozean vorhersagen zu können. Grundsätzlich begrenzt die Verwendung natürlicher unabhängiger Gemeinschaften, anstelle der direkten Manipulation von Biodiversität, die Möglichkeit für mechanistische Erklärungen. Ich schlage vor, das Problem in künftigen Studien zu überwinden, indem eine bekannte

Ursprungsgemeinschaft verwendet wird, in der Biodiversität (z.B. der Verlust oder die Verteilung gegebener Eigenschaften) in nicht zufälliger Weise manipuliert wird.



Pico, Azores – vulcão.

Introduction

The role of phytoplankton

Phytoplankton harbour an extremely diverse, polyphyletic group of microscopic photosynthetic protists (algae) and cyanobacteria. Accounting for roughly half the production of organic matter on Earth (Field et al. 1998), marine phytoplankton affect the abundance and diversity of marine organisms, drive marine ecosystem functioning, and set the upper limits to fishery yields (Chassot et al. 2010). Furthermore, phytoplankton strongly influence climate processes (Murtugudde et al. 2002) and biogeochemical cycles (Roemmich & McGowan 1995, Sabine et al. 2004), particularly the carbon cycle. Laws et al. (2000) showed that acting as biological carbon pump, phytoplankton and other organisms in the sunlit layer export up to 15% of the organic material produced each year to the deep sea, where about 0.1% of it gets buried in the sediment.

Major taxonomic groups of phytoplankton can be classified into distinct functional groups (Iglesias-Rodríguez et al. 2002) with unique biogeochemical signatures. Examples par excellence among marine phytoplankton are coccolithophores (Prymnesiophyceae) distinguished by delicate calcium carbonate platelets (coccoliths). Due to their worldwide distribution and their capacity to form extensive blooms when light and nutrient conditions are favourable, coccolithophores make an important contribution to carbon fixation (Tyrrell & Merico 2004) and play a key role in biogeochemical cycles (Sikes & Fabry 1994). Since major functional groups have different requirements and modes of acquisition of elements (Falkowski et al. 2004), such as carbon, nitrogen, and phosphorous, the composition of phytoplankton communities profoundly affects the biogeochemical cycling of these elements. If nothing else, phytoplankton are an excellent model system to address fundamental ecological questions due to their small size, short generation times, and large population numbers.

Biodiversity and ecosystem functioning

Biodiversity has often been used as a synonym for species richness (the number of species present), but different components of biodiversity (e.g. richness, relative abundance, composition, presence/absence of key species) can have different effects on ecosystem properties. Furthermore, biodiversity is not limited to species diversity, but also encompasses genetic diversity and functional diversity, the latter of which combines different species according to their functional traits. Ecosystem properties include both sizes of compartments (e.g. pools of materials such as carbon or organic matter) and rates of processes (e.g. fluxes of materials and energy among compartments). Ecological experiments, observations, and

theoretical developments show that ecosystem properties depend greatly on biodiversity in terms of the functional characteristics of organisms present in the ecosystem and the distribution and abundance of those organisms over space and time (Kinzig et al. 2002, Loreau et al. 2002). Species effects act in concert with the effects of climate, resource availability, and disturbance regimes in influencing ecosystem properties.

The mechanisms behind biodiversity effects can be broadly grouped into two types. First, the complementarity effect, which posits that niche differentiation or positive interactions in a community lead to increased total resource use. In particular, resource partitioning (Tilman et al. 1997) or facilitation among species (Cardinale et al. 2002) can increase the performance of communities above that expected from the best performing monoculture (referred to as transgressive overyielding). Second, the selection effect posits that the relationship between biodiversity and ecosystem functioning is determined by selective processes, such as interspecific competition, which cause the dominance of one very effective species driving ecosystem functioning. However, community overyielding due to selection effects never exceeds the performance of the best performing monoculture, but rather the performance of the average monoculture (Loreau & Hector 2001). Additionally, Fukami et al. (2005) demonstrated the necessity of taking historical perspectives of community assembly into account when testing for the effects of community composition on ecosystem functioning. These priority effects occur when the first colonizer of a patch gains numerical advantage such that it can exclude later colonists by monopolizing shared resources. Their results showed that even a small initial assembly effect on community composition can result in large variations in ecosystem functioning if the compositional difference is due to one or few functionally important species dominating different communities. Alternatively, community assembly may be important to community composition, but not to ecosystem functioning if variation in initial community assembly results in compositionally divergent, but functionally convergent, community structure. Since phytoplankton seasonal succession is often not a start from zero abundance a lack of attention to the role of initial community composition might miss important mechanisms.

Variation in sensitivity within functional groups has important implications for ecosystem responses. Functional redundancy in a community is a potential buffering mechanism when certain species are not able to deal with new environmental constraints. That is, many of the subdominant species are analogues of the dominants regarding the ecosystem functions they perform, but differ in terms of their capabilities to respond to environmental stress and disturbance. Thus, under changing conditions, ecosystem functioning can be maintained when subdominant species are able to substitute for the loss or decline of dominants (Walker et al. 1999, Niklaus et al. 2001).

Biodiversity in a changing world

Humans are altering the composition of biological communities through a variety of activities that increase rates of species invasions and species extinctions (Lotze 2006), at all scales, from local to global. These changes in components of the Earth's biodiversity cause concern for ethical and aesthetic reasons, but they also have a strong potential to alter ecosystem properties and the goods and services they provide to humanity.

Since the industrial revolution, atmospheric carbon dioxide (CO_2) increased from 280 to 380 µatm and is expected to further increase to 700 µatm by the year 2100 (Meehl et al. 2007). Until now, the oceans have absorbed approximately one-third of the carbon dioxide produced by human activities (Sabine et al. 2004). The uptake of anthropogenic CO_2 increases aqueous CO_2, bicarbonate (HCO_3^-), and hydrogen (H^+) ion concentrations, while decreasing the concentration of carbonate ions (CO_3^{2-}). Increasing H^+ concentrations have acidified the surface layers of the ocean, with an overall decrease since the pre-industrial period of 0.1 pH units (Caldeira & Wickett 2005). The reduction in pH together with the alterations in fundamental chemical balances is commonly referred to as ocean acidification. Ocean acidification represents a pervasive environmental change that is predicted to affect a wide range of species (Feely et al. 2004, Kroeker et al. 2010), yet our understanding of the emergent ecosystem impacts is very limited. Changes in carbonate chemistry can act both as fertilizer in case CO_2 is a limiting resource and as stressor. Based on a meta-analytic approach, Kroeker et al. (2010) revealed that calcifying organisms generally exhibit larger negative responses to ocean acidification than non-calcifying organisms. Nearly all marine phytoplankton possess inorganic carbon-concentrating mechanisms (CCM) to support photosynthetic carbon fixation at the concentrations of CO_2 present in ocean surface waters (Reinfelder 2011). Active transport of inorganic carbon by CCM is thought to account for a significant portion of cellular energy expenditure (Raven 1991). Savings from CCM down-regulation are likely to be responsible for possible acclimation of oceanic phytoplankton to rising CO_2 over the next century. Allocation of energetic savings to carbon fixation is most likely to occur under conditions where growth is limited by energy gain. In this case the energy savings from down-regulation of the CCM upon increasing ambient CO_2 concentrations could, thus, increase primary productivity by a few percent (Hopkinson 2011).

Reorganization of communities owing to altered environmental conditions involves changes in the physiology of individual species (physiological change), changes in the genetic composition of species (evolutionary change), and changes in species composition of communities (ecological change). Changes in community composition on the ecological and evolutionary level occur if the individual species have shifted out of their optima or exhausted their phenotypic plasticity (Ackerly 2003). Two most challenging questions largely remain

uncertain. Firstly, how much of the expected change in community functioning due to elevated CO_2 is owing to either changes in the physiology of individual species or in the relative abundance of species or is there a hint towards evolutionary adaptation? Secondly, how do effects of community composition on ecosystem functioning compare to direct effects of ocean acidification?

Thesis outline

This thesis is divided into three chapters. Each chapter represents an independent study addressing the effects of either exclusively community composition or the combination with ocean acidification on the biomass of experimental marine phytoplankton communities. This outline gives a brief overview of the motivation for the single experimental studies. In all three chapters I used experimental marine phytoplankton communities consisting of naturally coexisting species with a shared evolutionary history. Contrary to chapter III, where communities harboured several functional groups, in chapter I and II the communities were comprised only of coccolithophores.

Chapter I

The first chapter describes a microcosm experiment with communities consisting of four naturally co-occurring coccolithophore species. The aim of this study was to better understand how the functioning of the communities is affected by their structure. In particular, I tested whether varying initial dominance scenarios lead to different competitive outcomes and subsequently translate into altered community functioning. Initial community structure was manipulated by creating five different dominance scenarios: (1) all species contributing evenly to initial biomass, and (2-5) one of each specie contributing 4x that of the remaining species to total initial biomass. I explored biomass and carbon accumulation of the four species in monocultures and how their performance was reflected in the communities with the different initial dominance scenarios. Firstly, I predicted that in the monocultures, species differ in their carrying capacities. Secondly, I hypothesized that the initially dominant species retains its dominance due to priority effects. Lastly, I predicted that the functioning of the communities depends on community structure.

Chapter II

In the first study, I showed that community functioning was unaffected despite varying dominant species and different outcomes in the monocultures. In the second chapter, I conducted a second microcosm experiment with communities consisting of three of the four coccolithophore species used in the first study and explored the effect of initial community composition in combination with ocean acidification on community biomass. In particular, I tackled the question of how much of the expected change in community functioning due to elevated CO_2 is owing to either direct changes in the physiology of species or indirect ecological changes in the relative abundance of species. In order to complete the picture, I additionally indirectly tested for evolutionary adaptation to elevated CO_2. Firstly, I hypothesized that depending on species identity, elevated CO_2 has a direct physiological

effect on individual species during overwintering. Secondly, I predicted that elevated CO_2 alters community composition under overwintering conditions owing to physiological effects on individual species. Thirdly, I hypothesized that the indirect effect of altered initial community composition at the onset of a spring bloom due to elevated CO_2 during overwintering is equally important to ecosystem functioning than the direct physiological effect of elevated CO_2. Lastly, I predicted that species selected at elevated CO_2 exhibit lower performance when exposed back to ambient CO_2 in the ratio polycultures assemble after overwintering at ambient CO_2.

Chapter III

In chapter I and II, community functioning at bloom peak was affected neither by initial community composition nor ocean acidification. The communities in both studies however, consisted only of coccolithophores. In order to overcome this limitation, in chapter III, I used communities harbouring a variety of functional groups. The aim of this study was to tackle the question of how differences in initial community composition affect biomass build-up of a bloom compared to the direct effects of ocean acidification. I full-factorially exposed three compositionally different marine phytoplankton communities to two different CO_2 levels and examined the effects and relative importance (ω^2) of the two factors and their interaction on phytoplankton biomass at bloom peak. In particular, I firstly predicted that size and direction of the elevated CO^2 effect depend on initial community composition. I also hypothesiszed that both factors, initial community composition and elevated CO^2, are equally important to the regulation of phytoplankton biomass.

Cell isolation.

CHAPTER I

Initial dominance in coccolithophore communites affects community structure but does not translate into community functioning

Abstract

Climate change has the potential to profoundly influence the community structure and function of marine ecosystems. Prior to testing the consequences of altered environmental conditions on ecosystem functioning, it is first necessary to better understand how the functioning of an ecosystem is affected by its structure. Using phytoplankton communities with four naturally co-occurring coccolithophores including species of *Emiliania*, *Gephyrocapsa*, and *Calcidiscus* collected off the Azores, we experimentally tested whether varying initial dominance leads to different competitive outcomes and consequently affects community functioning, such as biomass and carbon accumulation. We manipulated initial community structure by creating five different dominance scenarios: (1) all species contributing evenly to total initial biomass, and (2–5) one of each species contributing 4× that of the remaining three species to total initial biomass. All four species were simultaneously grown in monocultures starting with the same total initial biomass as the communities. Monocultures differed significantly in total final biomass, particulate inorganic carbon, and particulate organic carbon content. Priority effects in the communities caused the initially dominant species to remain dominant during the stationary phase in three out of four cases. However, despite varying dominant species and different outcomes in the monocultures, community functioning was unaffected. We suggest that selective and facilitative effects are responsible for the equalization of community functioning. We conclude that monoculture experiments are not sufficient to predict whole-community responses, since species interactions can significantly alter the expected functional outcome.

Introduction

Shifts in biodiversity due to major anthropogenic stressors, such as climate change, modifications of global biogeochemical cycles, and alteration of species composition in food webs, are significant and growing (Millennium Ecosystem Assessment 2005). In most ecosystems, not only is the number of species changing, but also their relative abundance and there by dominance or evenness (Hillebrand et al. 2008). A meta-analysis of experimental studies by Walker et al. (2006) showed that warming increased dominance in plant communities within the tundra biome. Stachowicz et al. (2002) found a dominance shift to nonindigenous species due to warming in aquatic communities. Increasing CO_2 concentrations have also been found to alter dominance in grasslands (Niklaus et al. 2001). Changes in environmental conditions, such as pCO_2 and temperature in the oceans, have likewise induced dominance shifts in phytoplankton communities (Hare et al. 2007). Such alterations of biodiversity via changing species distributions most likely affect ecosystem functioning (i.e. biomass production, nutrient cycling, and habitat provision; Loreau et al. 2001, Hooper 2005). Whereas the effects of species loss on ecosystem processes have received broad attention (Hooper 2005, Cardinale et al. 2006, Hooper et al. 2012), the consequences of altered species dominance for emergent properties of communities and ecosystems are poorly investigated (Hillebrand et al. 2008). Evenness often changes more rapidly in response to anthropogenic stressors or altered environmental conditions than species richness. As such, altered patterns of evenness might lead to rapid changes in ecosystem functions before species are actually driven to extinction (Chapin et al. 2000). Understanding the underlying mechanisms of ecosystem functioning is important from two perspectives: first, for better understanding of ecological theory in order to classify, interpret, and predict the world around us; and second, for the development of solutions to environmental issues, such as mitigating negative effects of carbon emissions (Heimann & Reichstein 2008).

However, it remains challenging to resolve the effects of altered community structure on ecosystem functioning because consequences are often idiosyncratic and difficult to predict (Heimann & Reichstein 2008). In nature, priority effects can have major ramifications on community development (Young et al. 2001, Fukami et al. 2005) but have been rarely studied. They occur when the first colonizer of a new habitat gains a numerical advantage such that it can exclude later colonists by monopolizing shared resources. Fukami et al. (2010) demonstrated the necessity of taking historical perspectives of community assembly into account when testing for the effects of community structure on ecosystem functioning. Their results showed that even a small initial assembly effect on community structure can result in large variations in ecosystem functioning if the compositional difference is due to

one or a few functionally important species dominating different communities. Alternatively, community assembly may be important to community structure, but not to ecosystem functioning if variation in initial community assembly results in compositionally divergent, but functionally convergent, community structure.

Observed responses of ecosystem processes to changes in community structure or diversity can be broadly grouped into two types (Loreau & Hector 2001). First, the complementarity effect, which posits that resource partitioning or positive interactions in a community lead to increased total resource use. In particular, niche differentiation or facilitation between species can increase the performance of communities above that expected from the best performing monoculture (referred to as transgressive overyielding). Second, the selection effect posits that the relationship between biodiversity and ecosystem functioning is determined by selective processes, such as interspecific competition, which cause the dominance of one very effective species driving ecosystem functioning. However, community overyielding due to selection effects never exceeds the performance of the best performing monoculture, but rather the performance of the average monoculture (Loreau & Hector 2001).

In the present study, we used a model system of four marine, unicellular, calcifying phytoplankton species (coccolithophores) to experimentally test whether varying initial dominance scenarios translate into altered community functioning. Coccolithophores are distinguished by calcium carbonate plates refer red to as coccoliths. Due to their worldwide distribution and their important contribution to carbon fixation, coccolithophores play a key role in global biogeochemical cycles (Sikes & Fabry 1994). In particular, *Emiliania huxleyi* has the capacity to form extensive blooms in both coastal and oceanic waters (Brown & Yoder 1994) when water conditions are favorable. *E. huxleyi* blooms have been recorded to achieve cell concentrations of up to 115 million cells l^{-1} (Berge 1962). However, the particular set of conditions that leads to these blooms is not fully understood. Bottom-up factors (shallow mixed layer, high light, and high N:P ratios) and top-down factors (reduced grazing) have all been implicated as important for the development of *E. huxleyi* blooms (Tyrrell & Merico 2004).

In this study, we explored biomass and carbon accumulation of the four species in monocultures and how their performance is reflected in communities with different initial dominance scenarios. In particular, we tested the following hypotheses: (1) in monocultures, species differ in their carrying capacities. In particular, *Emiliania huxleyi* was expected to show the highest carrying capacity, which corresponds to massive blooms occurring in nature. (2) In communities, varying initial dominance scenarios affect the competitive outcome and consequently community structure. More precisely, the initially dominant species is also expected to retain its dominance due to priority effects. (3) Community

functioning depends on community structure. In particular, we expected communities dominated by *E. huxleyi* to possess the highest carrying capacities due to selection effects.

Methods

Study organsims

Four naturally co-occurring coccolithophore species, i.e. *Gephyrocapsa oceanica* (A), *G. muellerae* (B), *Calcidiscus quadriperforatus* (C), and *Emiliania huxleyi* (D) were used in the experiment. Cultures originated from strains that were isolated in April 2010 from waters off Faial Island (Azores, North Atlantic). The average cell biovolume of the four species was clearly different. Biovolumes were based on the spherical shape of the cells as described by Hillebrand et al. (1999), and calculated by determining the diameters for each species with a Z2™ COULTER COUNTER® prior to the experiment. The measured diameters resulted in a volume of 91 μm^3 for *E. huxleyi*, 99 μm^3 for *G. muellerae*, 205 μm^3 for *G. oceanica*, and 1920 μm^3 for *C. quadriperforatus*.

Experimental design

Initial community structure was manipulated by creating five different dominance scenarios (A dominant, B dominant, C dominant, D dominant, ABCD even). All four species were simultaneously grown in monocultures; this design allowed for quantitative comparisons between monocultures and communities. Each treatment was replicated four times resulting in 36 experimental units that comprised 2 L polycarbonate bottles randomly distributed across 4 climate cabinets.

At the onset of the experiment, 8 $\mu mol\ L^{-1}$ nitrate and 0.5 $\mu mol\ L^{-1}$ phosphate were added to 100 L of North Sea water with a salinity of 32 psu. These nutrient concentrations corresponded to a molar ratio of dissolved nitrogen to dissolved phosphate of 16:1. This reflects the prevailing oligotrophic nutrient regime across the study area that was measured while isolating the study organisms in April 2010. Vitamin and trace metal concentrations corresponded to 1/10 of a common f/2 medium (Guillard 1975). Initial pCO_2 and total alkalinity represented 380 ppm and 2330 $\mu mol\ kg^{-1}$, respectively. After sterile filtration (0.2 µm pore size), the water was transferred into the experimental units.

The differences in cell biovolume among the four species were balanced by starting each treatment with the same total initial biovolume, corresponding to a total initial biomass, of 153 600 $\mu m^3\ mL^{-1}$. Following field observations of phytoplankton community structure before the onset of a spring bloom (S. Jaschinski pers. comm.), dominance of species in the respective treatments was manipulated by adding the desired dominant species in a 4× higher

concentration than the other three remaining species. In the even treatment, each species contributed 25% to total initial biomass.

Cultures were exposed to 16°C and a light intensity of 130 µmol m^{-2} s^{-1} following a 16:8 h light:dark cycle. In order to limit sedimentation during the experiment, bottles were carefully rotated three times a day, each time with 15 rotations. The experimental duration was between 9 and 15 d. To ensure that cultures were sampled at the same state of growth, the exact development of each culture was determined by a sigmoidal growth model (see below).

Sampling and response variables

Cell abundance *(n)* and size *(d)* were determined every day with a Z2™ COULTER COUNTER®. The decision to terminate a culture was based on a statistically significant fit to the growth model:

$$nt = a \times \{1+[(a - b)/b] \times e^{(-\mu \times t)}\}^{-1} \quad \text{(I-1)}$$

where n_t is the number of cells after t days, a is the maximum cell abundance (carrying capacity), b is the starting cell number, and µ is the growth rate. The first day on which the growth curve of a culture significantly fitted the model, i.e. reached the stationary phase (carrying capacity), where outcome of interspecific competition is mostly pronounced, was defined as the first of 3 d in the stationary phase, after which the cultures were terminated. Final cell abundance and cell size were used to calculate total biovolume (= $n \times 1/6 \times \pi \times d^3$) after Hillebrand et al. (1999) as a measure of total biomass.

Community composition was determined every day by scanning electron microscopy (SEM) using a Phenom G2 pure desktop SEM. Samples were filtered through Whatman Nuclepore™ track-etched polycarbonate membranes (0.8 µm pore size, 25 mm Ø), dried, and analyzed. In total, 1000 cells $sample^{-1}$ were identified to calculate the relative abundance of each species. Additionally, the diameters of 25 randomly chosen cells per species were measured and used to calculate the average cell biovolume of each species. Cell abundance, relative abundance of each species, and the corresponding cell biovolume were used to calculate absolute biomass of each contributing species in the communities.

At the end of the experiment, samples for dissolved inorganic nutrients, total particulate carbon (TPC), and particulate organic carbon (POC) were taken (Whatman GF/F filters, 25 mm Ø). For the latter, the particulate inorganic carbon (PIC) was removed by exposing filters containing TPC to fuming hydrochloric acid for 12 h. Before measurement, filters were dried at 60°C, folded, packed in tin cups, and subsequently analyzed with an elemental analyzer with a thermal conductivity detector (FlashEA 1112). PIC was determined by subtracting POC from TPC. Discriminating between POC and PIC allows disentanglement of the

proportions of biologically fixed carbon that either return back to the carbon cycle through remineralization and/or decomposition (POC) or contribute to carbon export from the surface to the ocean sediments (PIC). Dissolved inorganic phosphate and nitrate were measured with continuous flow analyses following Hansen & Koroleff (1999) using a SKALAR SANPLUS auto-analyzer system.

Statistical analyses

Prior to statistical analyses, data were tested for normality and homogeneity of variances. If data were not normally distributed or variances were not homogeneous, data were square-root transformed. Addressing hypothesis 1, we tested the effect of species identity (SI) on total biomass, TPC, POC, and PIC by calculating a 1-way analysis of variance (ANOVA) among the monocultures (4 levels). To determine which species significantly differed from each other, all possible pairs of means were compared by Tukey's HSD test afterwards.

In addition, the final absolute biomass of the initially dominant species within each of the different communities, excluding the even treatment, was tested against the yield of the respective monoculture with a Student's *t*-test (i.e. A in Abcd versus A in monoculture, etc.). Addressing hypothesis 3, we tested the effects of initial community structure (ICS) on total biomass, TPC, POC, and PIC by calculating a 1-way ANOVA among the communities with 5 levels of dominance. The occurrence of overyielding due to selection effects was tested by calculating a 1-way ANOVA with 6 levels including the average monoculture in addition to the 5 different communities. To determine which communities significantly differed from the average monoculture, each of the dominance scenarios was compared to the average monoculture by Fisher's protected LSD test afterwards.

The consistency of TPC, POC, and PIC with total biomass was confirmed by a positive Pearson correlation coefficient in both the monocultures and the communities (monocultures: $r_{TPC} = 0.89$, $r_{POC} = 0.92$, $r_{PIC} = 0.74$, $p < 0.01$ for all, $N = 16$; communities: $r_{TPC} = 0.86$, $r_{POC} = 0.77$, $r_{PIC} = 0.89$, $p < 0.01$ for all, $N = 20$). Therefore, we consider biomass as the appropriate response variable to mechanistically explain our findings.

Observed differences in the duration until nutrient depletion between the communities and their respective monocultures (e.g. abcD versus D) were confirmed with a Student's *t*-test comparing the number of days that it took a culture to deplete nutrients.

Results

Total yields of monocultures

Species identity significantly affected total biomass, TPC, and POC (Table 1, Fig. 1). The four species differed significantly in total biomass, except *Calcidiscus quadriperforatus* (C)

from *Emiliania huxleyi* (D). *Gephyrocapsa oceanica* (A) was the best performing species (i. e. reached the significantly highest carrying capacity; Tukey HSD: A versus B, C, D: $p < 0.001$). The second highest yielding species was *G. muellerae* (B; Tukey HSD: B versus C: $p < 0.001$; B versus D: $p < 0.05$; Fig. 1). *C. quadriperforatus* (C) and *E. huxleyi* (D) were both lower-yielding species (Fig. 1). Regarding TPC and POC accumulation, both *Gephyrocapsa* species (A,B) were significantly higher yielding than *C. quadriperforatus* (C) and *E. huxleyi* (D); (Tukey HSD: $p < 0.01$). PIC accumulation was not significantly affected by species identity (Table 1).

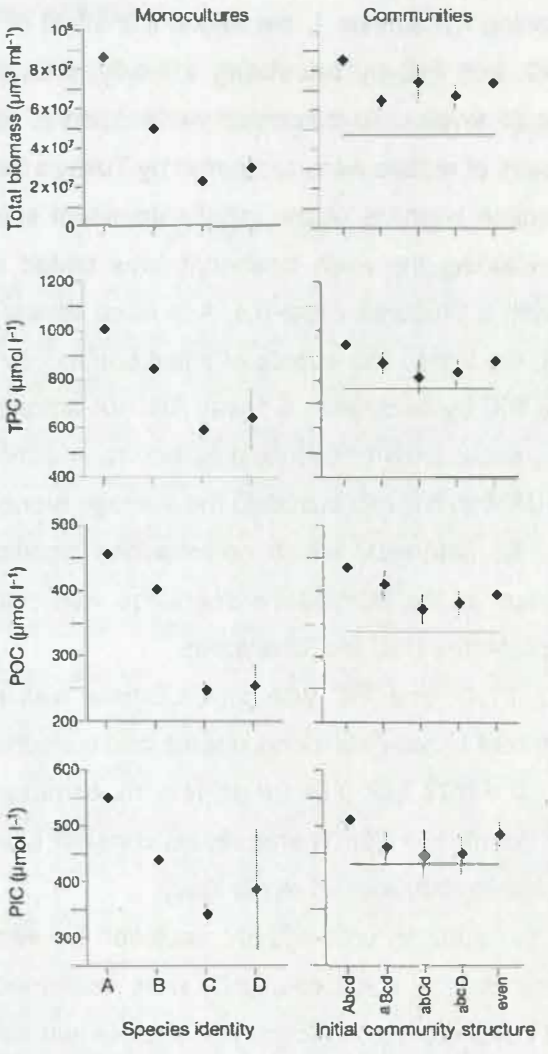


Fig. I-1. Total biomass (calculated as biovolume), total particulate carbon (TPC), particulate organic carbon (POC), and particulate inorganic carbon (PIC) in monocultures and communities at the end of the experiment. Capital letters on the *x*-axes of the community plots indicate the initial dominant species. Species identities are (A) *Gephyrocapsa oceanica*, (B) *G. muellerae*, (C) *Calcidiscus quadriperforatus* and (D) *Emiliania huxleyi*. Black lines refer to the overall mean of the average monoculture. Error bars are ± SE.

Table I-1. Results of 1-way ANOVAs testing the effects of species identity (SI) and initial community structure (ICS) on total biomass, total particulate carbon (TPC), total particulate organic carbon (POC), and particulate inorganic carbon (PIC) among monocultures (4 levels), among communities (5 levels), and among communities plus the average monoculture (6 levels). Shown are degrees of freedom (df_{model}, $df_{residual}$), the variance explained by the model (R^2), the *F* ratio, and the probability that the variation is random. Values in **bold** are significant at $p < 0.05$

	Monocultures					Communities					Communities plus average monoculture			
Response Variable	Factor	df	R^2	*F*	p	Factor	df	R^2	*F*	p	df	R^2	*F*	p
Total biomass	SI	3, 12	0.92	59.07	**<0.001**	ICS	4, 15	0.21	2.23	0.12	5, 30	0.26	3.47	**<0.05**
TPC	SI	3, 12	0.60	8.48	**<0.01**	ICS	4, 15	0.02	1.08	0.40	5, 30	0.00	1.01	0.43
POC	SI	3, 12	0.86	32.56	**<0.001**	ICS	4, 15	0.18	2.02	0.14	5, 30	0.07	1.53	0.21
PIC	SI	3, 12	0.24	2.62	0.10	ICS	4, 15	0.06	0.74	0.58	5, 30	−0.06	0.59	0.71

Final community structure

Analyses of the relative biomass of each species over time in the different communities showed that in the cases of *Gephyrocapsa oceanica* (A), *G. muellerae* (B), and *Emiliania huxleyi* (D) being the initially dominant species, these species remained dominant until stationary phase. Their final contributions to total biomass accounted for 57, 50, and 75%, respectively (Fig. 2). In the case of *Calcidiscus quadriperforatus* (C) being the initially dominant species and in the even treatment, *Emiliania huxleyi* (D) ultimately dominated these communities. Here, the relative contribution of *E. huxleyi* (D) to total biomass represented 40 and 48%, respectively (Fig. 2).

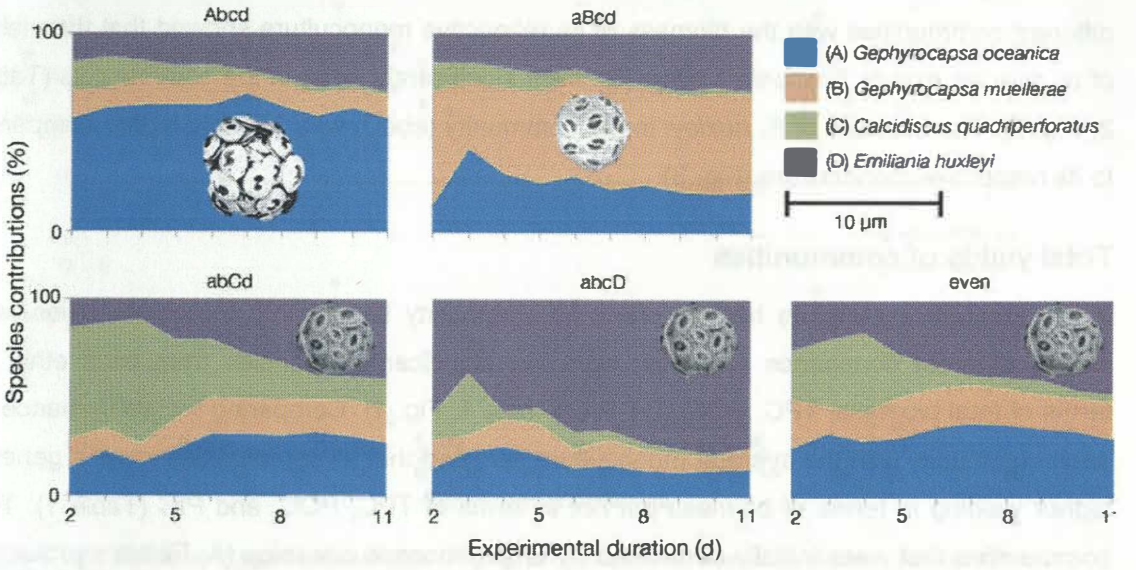


Fig. I-2. Relative community compositions over time in the different communities (capital letters in the headings indicate the initial dominant species, and scanning electron micrographs illustrate the dominant species at the end of the experiment).

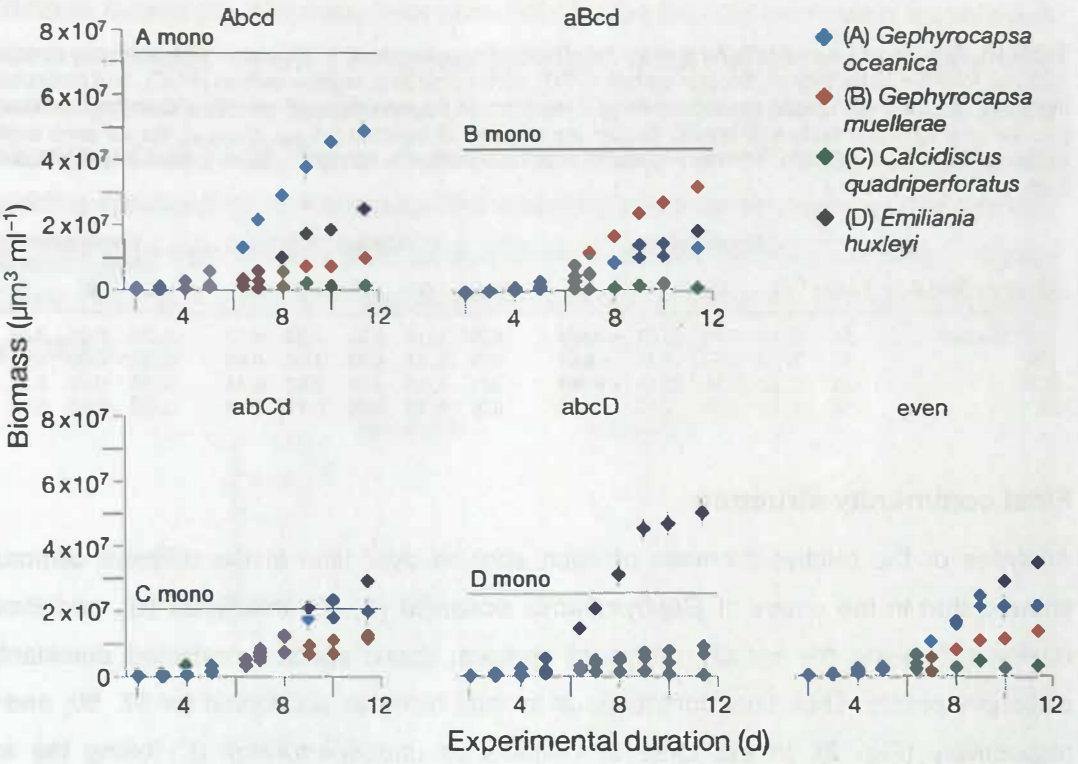


Fig. I-3. Absolute biomass (calculated as biovolume) of each species in the different communities (capital letters in the headings indicate the initial dominant species). Black lines refer to the mean of the dominant species in monocultures (mono) at the end of the experiment. Error bars are ± SE.

Comparing the final absolute biomass of the initially dominant species within each of the different communities with the biomass of its respective monoculture showed that the yields of all species except *Emiliania huxleyi* (D) were significantly lower in the communities (Table 2, Fig. 3). The biomass of *E. huxleyi* in the community (abcD) was 1.7- fold higher compared to its respective monoculture (Fig. 3).

Total yields of communities

Initial dominance structure had no effect on community functioning; the communities with initially different dominance scenarios were not significantly dissimilar from each other in terms of total biomass, TPC, POC, and PIC (Table 1, Fig. 1). Comparing the performance of each community with the average monoculture revealed that all communities were in general higher yielding in terms of biomass but not in terms of TPC, POC, and PIC (Table 1). The communities that were initially dominated by *Gephyrocapsa oceanica* (A; Fisher's protected LSD test: $p < 0.01$) and *Calcidiscus quadriperforatus* (C; Fisher's protected LSD test: $p < 0.05$) as well as the initially even communities (Fisher's protected LSD test: $p < 0.05$) were significantly higher yielding than the average monoculture. The communities that were initially dominated by *G. muellerae* (B) and *Emiliania huxleyi* (D) were both marginally higher

than the average monoculture (Fisher's protected LSD test: p = 0.091 and 0.063, respectively).

Dissolved inorganic nitrogen and phosphorous

The amount of phosphorus taken up was similar in all communities and was depleted after 11 d (Fig. 4). The monocultures of *Gephyrocapsa oceanica* (A), *G. muellerae* (B), and *Calcidiscus quadriperforatus* (C) followed the same pattern. *Emiliania huxleyi* (D) in monoculture showed the highest affinity for inorganic phosphorus compared to the other 3 species, leading to fastest depletion after 8 d (Fig. 4). Thus, in the communities which were initially dominated by *E. huxleyi* (abcD), inorganic phosphorus was available significantly longer (on average 2 d) than in the *E. huxleyi* (D) monocultures (*t*-test: df = 6, $t = -3.0$, $p < 0.05$; Fig. 4).

Nitrogen was taken up similarly in all the communities and depleted after 8 d except in the community that was initially dominated by *Emiliania huxleyi* (D), where nitrogen was available until Day 10 (Fig. 4). The depletion of nitrogen followed the same pattern in the monocultures except the monoculture of *Calcidiscus quadriperforatus* (C). Here, nitrogen was available until Day 14.

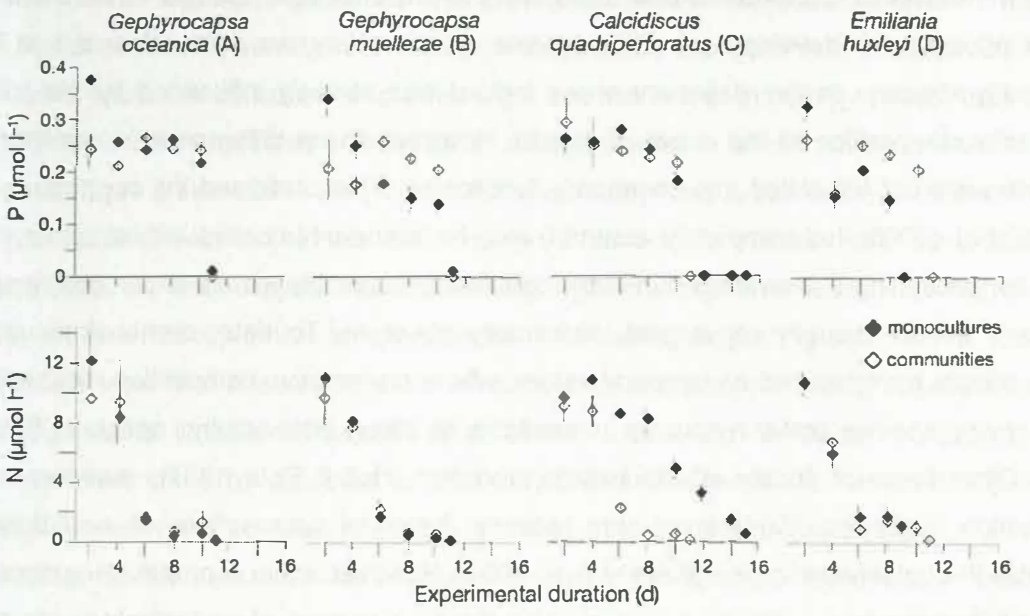


Fig. I-4. Total dissolved inorganic phosphorus (P) and nitrogen (N) over time in the different monocultures and communities. Headings indicate the species (in monoculture) and the initial dominant species (in the community). The quantification limit is 0.1 µmol L^{-1} and indicates the minimum concentration at which we can be confident that the numerical result is accurate. Data below the quantification limit are assumed to be 0.

Discussion

The four species clearly differed in their carrying capacities as demonstrated in the monocultures (supporting hypothesis 1). However, contrary to our expectations, the highest carrying capacity was not established by *Emiliania huxleyi* but by *Gephyrocapsa oceanica*. In general, varying initial dominance scenarios in the communities led to different competitive outcomes that were consequently reflected in community structure (supporting hypothesis 2). More precisely, in three out of four cases, the initially dominant species (i.e. *G. oceanica*, *G. muellerae*, and *E. huxleyi)* remained dominant due to priority effects. However, these differences in community structure were not translated into community functioning (rejecting hypothesis 3), as could be expected according to different outcomes in the monocultures. That is, the overall performance of the communities was equal. In general, all communities overyielded the average monoculture in terms of biomass due to selective and facilitative effects.

Community structure and functioning

Prior to the onset of phytoplankton spring blooms, alongside a variety of environmental conditions, the initial composition and distribution of phytoplankton species is assumed to greatly influence the development of the blooms. In this study, we demonstrated that the community structure in the stationary phase indeed was strongly influenced by the initial community composition at the onset of growth. However, these differences in community structure were not translated into community functioning. This confirmed the suggestion by Fukami et al. (2005) that community assembly may be important to community structure, but not to its functioning if the variation in initial community assembly results in compositionally divergent, but functionally convergent, community structure. To date, demonstrations of priority effects have focused on temporal scales, where pre-emptive competition means that early-arriving species make resources unavailable to other, later-arriving species (Tilman 1988). Other forms of priority effects include predation (Holt & Polis 1997), environmental modification (Knowlton 2004) and, more recently, historical perspectives of evolutionary assembly through diversification (Fukami et al. 2007). However, initial community structure is still not fully taken into account when explaining the development of phytoplankton blooms. The initial community structure is no less important to the functioning, spatial extent, and duration of phytoplankton spring blooms than are the abiotic conditions.

In general, the functioning of the present communities was shaped by selective and partly by facilitative processes. That is, functioning in all communities was driven by single effective species, resulting in total community performances overyielding the average monoculture. Moreover, our results suggest that different mechanisms were responsible for equalizing the

functioning among the communities despite distinct expectations from the monocultures and explicit differences in community composition. In the case of *Gephyrocapsa oceanica* or *G. muellerae* being the dominant species, community functioning was driven only by classical selection effects. That is, the dominant competitor was the driving species for total community performance, and thus community functioning did not deviate from the respective dominant species' monoculture. In the communities that contained *Emiliania huxleyi* as initially dominant, its performance was facilitated by the presence of at least one of the other three species. The facilitation effect caused the *E. huxleyi* dominated communities to clearly perform better than was expected from the *E. huxleyi* monocultures (Fig. 3). Consequently, the differences in structure among the communities leveled out in functioning. The experimental design used in the present study, however, did not allow us to determine which particular species or set of species was responsible for the facilitation effect.

Though highly speculative, a possible mechanistic explanation for the facilitative effect might be found in different phosphorus sources between the monocultures of *Emiliania huxleyi* and the communities which were initially dominated by *E. huxleyi.* Inorganic phosphorus was available significantly longer in the *E. huxleyi*-dominated communities compared to the respective monocultures, which suggests that *E. huxleyi* used an alternative phosphorus source such as organic phosphorus. This allowed for almost one more cell division, resulting in a higher population density of *E. huxleyi* than could be expected from its monocultures. *E. huxleyi* is known to be superior to other algal species under extremely phosphorus-limited conditions, which is reflected in massive *E. huxleyi* blooms in phosphorus-depleted areas or seasons (Egge & Heimdahl 1994, Tyrrell & Taylor 1996). This is caused by its high affinity for inorganic phosphorus on the one hand, and on the other by its possession of several alkaline phosphatase (APase) enzyme systems making organic phosphorus available (Riegman et al. 2000, Xu et al. 2010), which is, for example, provided by degrading cells. For *E. huxleyi* it could be shown that relevant APases were induced and enzyme activity rapidly increased with phosphorus limitation (Riegman et al. 2000, Xu et al. 2010). So far, no expression of these APases could be detected in other phytoplankton species (Xu et al. 2010). APase activity can enhance population growth by up to 90% through organic phosphorus uptake depending on the concentration of available organic phosphorus. Moreover, in chemostat experiments, organic phosphorus uptake rate correlated negatively with growth rate and positively with organic phosphorus concentration (Riegman et al. 2000). This means that APase activity becomes increasingly important during the stationary phase (Xu et al. 2010), i.e. phytoplankton bloom peak, when more organic phosphorus is available through degrading cells. Interpreting this in the context of our study suggests that in the *E. huxleyi*-dominated communities, organic phosphorus was provided by degrading cells of the subdominant species. This source of organic phosphorus was not available in *E. huxleyi*

monocultures and thus led first to faster depletion of inorganic phosphorus and second to significantly lower cell abundance. Strictly speaking, facilitation describes species interactions that benefit at least one of the participants and harm neither (Stachowicz 2001). Thus, our finding is not a true facilitation effect because the subdominant species experienced reduced performance. This is likely due to the general competitive advantage of *E. huxleyi* in both inorganic and organic phosphorus up take. However, overyielding can be taken as an indicator for facilitation (Hector et al. 2009).

The communities that were initially dominated by *Calcidiscus quadriperforatus* or evenly assembled and were finally dominated by *Emiliania huxleyi* likewise did not differ from the other communities in total functioning and also overyielded the average monoculture. In both communities, final dominance of *E. huxleyi* was less pronounced compared to the communities that were initially dominated by *E. huxleyi* and the final contribution of each species was more even. However, in these communities, *E. huxleyi* was likewise never outperformed by its respective monoculture, suggesting that it benefitted from the presence of the other species via the suggested phosphorus uptake strategies. Thus, the dominance of *E. huxleyi* in the presence of subdominant species most likely led to an increased resource use efficiency along the altered N:P gradient. Overall, the different mechanisms that were triggered by relatively small compositional differences during community assembly led to communities that showed either lower or higher carrying capacities of their dominant species than could be expected from the corresponding monocultures. Thus, independent of their structure, the overall functioning in the communities equalized.

Implications for changing oceans

The anthropogenically induced rise in atmospheric pCO_2 leads to changes in seawater carbonate chemistry known as ocean acidification, which is considered to have major effects on calcifying organisms (Caldeira & Wickett 2005, Orr et al. 2005). In the present experiment, we used model organisms that were previously used to address hypotheses testing the consequences of rising atmospheric pCO_2 mostly for the physiological performance of these organisms (e.g. Riebesell et al. 2000, Langer et al. 2006). In this study, we focused on ecological mechanisms pointing to the fact that different interaction effects between species make functional outcomes hard to predict. Therefore, multifactorial approaches combining community structure and environmental stress might have unexpected implications for population responses to global climate change. This has the potential to produce more realistic results than derived from pure monoculture experiments.

Dissolution of CO_2 in seawater results in increased concentrations of bicarbonate and hydrogen ions and therefore a decrease in seawater pH. This eventually leads to a decreasing availability of carbonate ions. Since the latter are crucial building blocks in

calcifying organisms, a rising atmospheric pCO_2 potentially leads to reduced calcification in marine organisms (Guinotte & Fabry 2008). Following physical laws, the solubility of CO_2 increases with decreasing temperature. Thus, calcifying organisms might primarily become impaired or even lost during the overwintering phase in temperate and polar latitudes. This might have severe consequences for spring bloom community compositions and consequent community functioning.

Previous studies have demonstrated that among calcifying phytoplankton there are species-specific differences in the response to changes in seawater carbonate chemistry. Among the coccolithophores, *Gephyrocapsa oceanica* (Langer et al. 2006) seems to be most prone to increasing pCO_2, whereas *Emiliania huxleyi* showed less sensitivity (Riebesell et al. 2000) or adaptive evolution in the long term (Lohbeck et al. 2012). Our results point to the fact that *E. huxleyi* may depend on other subdominant coccolithophore species to facilitate its performance and consequently allow the occurrence of massive *E. huxleyi* blooms. Assuming that *G. oceanica* would be responsible for the facilitative effect in our model system, losing this species due to direct negative physiological effects on changes in seawater carbonate chemistry would consequently lead to indirect negative effects of altered community structure on the performance of *E. huxleyi*. However, transferring this to natural communities and further disentangling the effects of community structure and abiotic stress such as rising pCO_2 requires the inclusion of more functional groups such as diatoms. The latter have been shown to initially dominate natural phytoplankton spring blooms until dissolved inorganic nutrients, primarily silicate and phosphorus, become limiting (Tyrrell & Merico 2004). Subsequent blooms of *E. huxleyi* are among other environmental factors triggered by low dissolved inorganic phosphorus concentrations and increased availability of dissolved organic phosphorus due to degrading diatoms. The great challenge predicting the effects of climate change, such as increasing sea surface temperature and pCO_2, on ecosystems is in understanding how altered species interactions will affect succession and extent of phytoplankton blooms.

To our knowledge, this study is among the first to mechanistically show that small differences in initial community composition can have unpredictable effects on community functioning, as species interactions are highly idiosyncratic, which can significantly alter the functional outcome expected from monocultures. This points to the likelihood that understanding changing community interactions along with global environmental change such as ocean acidification will uncover unexpected consequences for biogeochemical cycles.

Acknowledgements

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CHAPTER II

Physiologically induced shift in the composition of a coccolithophore community does not alter community functioning in response to ocean acidification – but is there a hint towards evolutionary adaptation?

Abstract

When testing the physiological effects of ocean acidification on ecosystem functioning it is necessary at the same time to understand its consequences for species composition. Completing the picture even further requires including the evolutionary effect that can be due to genotypic selection or new mutations. To date, the relative importance among these three possible responses to changing environmental conditions largely remains unknown. Using a model system of three naturally co-occurring coccolithophores including species of Emiliania and Gephyrocapsa we set out to experimentally explore how much of the expected change in community biomass due to elevated CO_2 is owing to either direct changes in the physiology of individual species or indirect ecological changes in the relative abundance of species. Additionally, we indirectly tested for evolutionary adaptation to elevated CO_2. We found that the performance among individual species during a simulated overwintering phase differed across both tested CO_2 levels. Owing to these physiological effects on individual species, elevated CO_2 altered the community composition during overwintering. Though, after initiating a spring phase we found contrary to our expectation neither a significant physiological effect nor an ecological effect of elevated CO_2 on biomass at bloom peak. However, biomass in those communities that were selected at elevated CO_2 during overwintering was lower when exposed back to ambient conditions hinting towards evolutionary adaptation. We suggest that the lacking effect on ecosystem functioning in this particular model system in response to elevated CO_2 may be caused by community reorganization due to evolutionary adaptation. However, to further unravel the interplay between physiological, ecological, and evolutionary effects of elevated CO_2 on ecosystem functioning it is necessary to include a variety of functional groups in future experiments.

Introduction

Marine phytoplankton are at the base of the pelagic food web and responsible for roughly half of the world's primary production (Falkowski 2012). They are also of global significance for climate regulation and biogeochemical cycling and act as a biological carbon pump that exports fixed carbon to the deep ocean (Doney et al. 2009). The uptake of anthropogenic CO_2 has acidified the surface layers of the oceans with an overall decrease since the pre-industrial period of 0.1 pH units (Caldeira & Wickett 2005). Associated with these changes in pH is a substantial decline in carbonate ion concentration and carbonate saturation (Feely et al. 2004). These changes in seawater chemistry commonly referred to as ocean acidification (Caldeira & Wickett 2003), can affect the composition of phytoplankton communities. Though calcifying taxa generally exhibit larger negative responses to ocean acidification than non-calcifying taxa (Kroeker et al. 2010, Kroeker et al. 2013), the sensitivity varies among species. Examples par excellence among marine phytoplankton are coccolithophores (Prymnesiophyceae), unicellular microalgae, distinguished by delicate calcium carbonate platelets (coccoliths). Due to their worldwide distribution and their capacity to form extensive blooms when light and nutrient conditions are favourable (Tyrrell & Merico 2004), coccolithophores make an important contribution to carbon fixation and play a key role in global biogeochemical cycles (Sikes & Fabry 1994). Previous studies on the effects of ocean acidification on coccolithophores focused predominantly on physiological responses of individual species and revealed variation among species (Riebesell et al. 2000, Langer et al. 2006).

Ecological experiments, observations, and theoretical developments show that ecosystem functioning depends greatly on biodiversity in terms of the functional characteristics of organisms present in the ecosystem and the distribution and abundance of those organisms over space and time (Kinzig et al. 2002, Loreau et al. 2002). The variation in sensitivity within functional groups has important implications for ecosystem responses. Functional redundancy in a community is a potential buffering mechanism when certain species are not able to deal with new environmental constraints such as elevated CO_2. That is, many of the subdominant species are analogues of the dominants regarding the ecosystem functions they perform, but differ in terms of their capabilities to respond to environmental stress and disturbance. Thus, under changing conditions, ecosystem functioning can be maintained when subdominant species are able to substitute for the loss or decline of dominants (Walker et al. 1999, Niklaus et al. 2001). *Emiliania huxleyi* is by far the most abundant species among coccolithophores on a global basis, dominating annual coccolithophore spring blooms such as those in the subtropic N. Atlantic with roughly 64% (Haidar & Thierstein 2001); subdominant coccolithophore species each contribute less than 2%.

Since the solubility of CO_2 increases with decreasing temperature and the $CaCO_3$ saturation state generally declines with increasing latitude (Caldeira & Wickett 2005, Orr et al. 2005), undersaturation of surface open ocean waters will be reached first in polar waters, as fossil fuel emission intensify ocean acidification in the future. Accordingly, the probability of coccolithophores to be impaired owing to elevated CO_2 in temperature latitudes is most likely to occur during overwintering. This in turn might hold profound consequences for initial spring bloom community composition. Empirical evidence from long-term studies suggests that the degree of overwintering plankton influences the seasonal dynamics in marine ecosystems, possibly associated with alterations in phenology or changes in community structure (Sommer & Lewandowska 2011). Such priority effects can have major ramifications on community functioning (Matthiessen & Hillebrand 2006, Fukami et al. 2007).

In a nutshell, a reasonable approach to explaining altered community functioning requires that research integrates changes in the physiology of individual organisms and ecological changes in species composition. Both must be considered simultaneously to understand community responses to ocean acidification in marine phytoplankton. Completing the picture even further requires including the evolutionary effect that is due to genetic responses.

In the present study, we used a model system of three coccolithophore species to experimentally explore how much of the expected change in community functioning (here: biovolume) due to elevated CO_2 is owing to either direct changes in the physiology of individual species or indirect ecological changes in the relative abundance of species. Additionally, we indirectly tested for evolutionary adaptation to elevated CO_2. In particular, we addressed the following hypotheses: (i) depending on species identity, elevated CO_2 has a direct physiological effect on individual species during overwintering. (ii) Elevated CO_2 alters community composition under overwintering conditions owing to physiological effects on individual species. (iii) The indirect effect of altered initial community composition at the onset of a spring bloom due to elevated CO_2 during overwintering is equally important to ecosystem functioning than the direct physiological effect of elevated CO_2. (iv) Communities selected at elevated CO_2 exhibit lower performance when exposed back to ambient CO_2 in the ratio polycultures assemble after overwintering at ambient CO_2.

Methods

Study organisms and culture media

Three naturally co-occurring coccolithophore species, i.e. *Emiliania huxleyi*, *Calcidiscus quadriperforatus*, and *Gephyrocapsa muellerae*, were used in the experiment. Cultures were founded by cell isolation from waters off Faial Island (Azores, N. Atlantic) in April 2010. The average cell biovolume clearly differed among the 3 species. Biovolumes were based on the

spherical shape of the cells as described by Hillebrand et al. (1999), and calculated after determining the average diameter for each species with a Z2™ COULTER COUNTER® (Beckman Coulter, Brea, USA) prior to the experiment. The measured diameters resulted in an average volume of 95 μm^3 for *E. huxleyi*, 118 μm^3 for *G. muellerae*, and 1067 μm^3 for *C. quadriperforatus*.

Artificial seawater (ASW) of 35 psu and a total alkalinity (TA) of 2345 $\mu mol\ kg^{-1}$ was produced as in Kester et al. (1967) and 0.2 µm sterile filtered (Sartorius Sartobran 300, Göttingen, Germany). The ASW was supplemented with 8 $\mu mol\ kg^{-1}$ nitrate and 0.5 $\mu mol\ kg^{-1}$ phosphate, corresponding to a molar nutrient ratio of 16:1 after Redfield (1958), trace metals and vitamins according to f/10 adapted by Guillard & Ryther (1962), 10 $nmol\ kg^{-1}$ selenium after Danbara & Shiraiwa (1999), and 10 $mL\ kg^{-1}$ sterile filtered North Sea water to exclude any limitations by micronutrients.

Prior to inoculation, the ASW medium was bubbled for 24h in parts with air ('*Non-acidified*', pCO2 ≈ 400 µatm) and in parts with air enriched with gaseous CO_2 ('*Acidified*', pCO_2 ≈ 2000 µatm) using a controlled CO_2 gas mixing system. Evaporation was minimized by gas wash bottles filled with MilliQ water to saturate gases with humidity. After CO_2 manipulations, ASW media were carefully pumped into the experimental units, comprising 2 L polycarbonate bottles, leaving a minimum headspace. For each pCO_2 level, two extra flasks were prepared for dissolved inorganic carbon (DIC) and total alkalinity measurements.

Experimental cultures were grown in a RUMED Light Thermostat 1201 (Rubarth Apparate GmbH, Germany). All culture flasks were manually rotated 15 times, thrice a day, in order to limit sedimentation.

Experimental design

Overwintering phase

During 28 days of overwintering, all 3 species were cultivated in monocultures and together in polycultures in both CO_2 levels, respectively (Fig. 1). Each treatment combination (culture x CO_2) was quadruplicated, resulting in 32 experimental units. Experimental cultures were cultivated at 10 °C (Silva et al. 2009) and a light intensity of 20 $\mu mol\ photons\ m^{-2}\ s^{-1}$ following a 8:16 light:dark cycle (adapted from Brock 1981). The differences in cell biovolume among the 3 species were balanced by starting each experimental culture with the same total initial biovolume of≈ 150 000 $\mu m^3\ mL^{-1}$. In the polycultures, each species contributed a third to total initial biovolume. Cell abundance and size were measured every 7 days using the Z2™ COULTER COUNTER®.

At the end of the overwintering phase, community composition in the polycultures was determined by scanning electron microscopy (SEM) using a Phenom G2 pure desktop SEM (LOT-QuantumDesign GmbH, Darmstadt, Germany). Samples were filtered through

Whatman Nucleopore™ track-etched polycarbonate membranes (0.8 µm pore size, 13 mm Ø), dried and analysed. In total, 1000 cells sample^{-1} were identified to calculate the relative abundance of each species. Additionally, the diameters of 25 randomly chosen cells per species were measured and used to calculate the average cell biovolume of each species. Cell abundance, relative abundance of each species, and the corresponding cell biovolume were used to calculate the absolute biovolume of each contributing species in the communities.

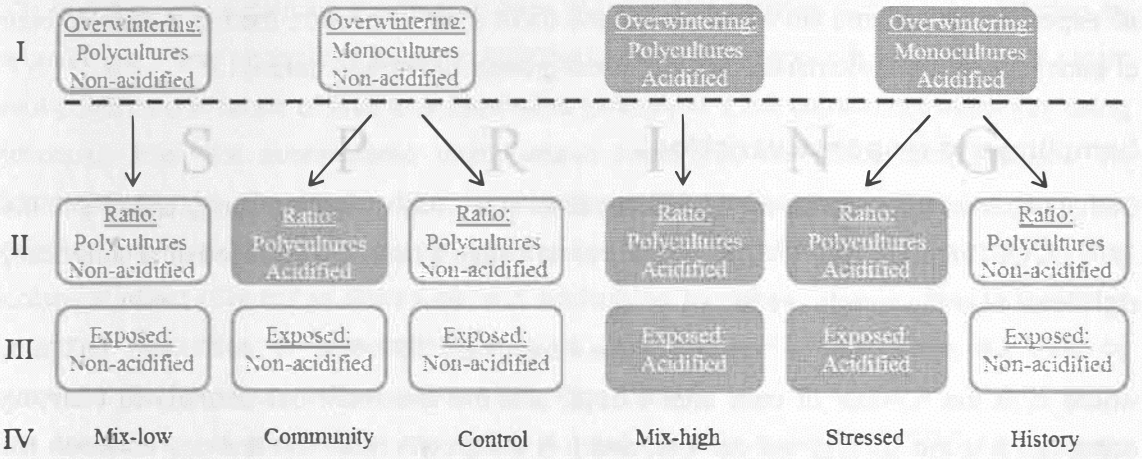

Fig. II-1. Experimental design: (I) overwintering phase: all 3 species were cultivated in monocultures and together in polycultures in both CO_2 levels, respectively (Non-acidified = white rectangles; Acidified = grey rectangles). (II) Ratios in which the three species were inoculated after overwintering; either directly from the polycultures or assembled from the monocultures (III) CO_2 levels, cultures were exposed to at the onset of spring. (IV) Treatment designations.

Spring phase

The spring phase was initiated by starting a new batch cycle (Fig. 1). During a 6 days lasting transition phase temperature and light intensity were gradually increased towards 16 °C (Silva et al. 2009) and 160 µmol photons m^{-2} s^{-1}, respectively. On day 6 the light:dark cycle was changed to 14:10 (adapted from Brock 1981). The polycultures grown under *Non-acidified* conditions during the overwintering phase were directly inoculated again into *Non-acidified* conditions ('*Mix-low*'; Fig. 1) and those grown under *Acidified* conditions during the overwintering phase directly again into *Acidified* conditions ('*Mix-high*'; Fig. 1). Additionally, we assembled communities in the species ratio observed in the polycultures after overwintering under *Non-acidified*/*Acidified* conditions using individuals from *Non-acidified*/*Acidified* overwintered monocultures and inoculated them into *Non-acidified*/*Acidified* conditions ('*Control*'/'*Stressed*'; Fig.1). In order to disentangle the pure ecological effect of elevated CO_2 from the combined physiological and ecological effect, we assembled communities in the species ratio observed in the polycultures after overwintering under *Acidified* conditions using individuals from *Non-acidified* overwintered monocultures and inoculated them into *Non-acidified* conditions ('*Community*'; Fig. 1). Furthermore, in

order to examine potential correlated responses to selection, we assembled communities in the species ratio observed in the polycultures after overwintering under *Non-acidified* conditions using individuals from *Acidified* overwintered monocultures and inoculated them into *Non-acidified* conditions (*History*; Fig. 1). Each treatment was quadruplicated, resulting in 24 experimental units. All experimental cultures started the spring phase with a total initial biovolume of ≈ 5000 $\mu m^3\ mL^{-1}$.

The duration of the spring phase to the end of the experiment was 27 days. To ensure that all experimental cultures were sampled at the same state of growth, the exact development of each culture was determined by a sigmoidal growth model (see below).

Sampling and response variables

Cell abundance (*n*) and size (*d*) were determined every day during the spring phase with the Z2™ COULTER COUNTER®. The decision to terminate a culture was based on a statistically significant fit to the growth model:

$$n_t = a \times \{1+[(a - b)/b] \times e^{(-\mu \times t)}\}^{-1} \quad \text{(II-1)}$$

where n_t is the number of cells after t days, a is the maximum cell abundance (carrying capacity), b is the starting cell number, and μ is the growth rate. The first day on which the growth curve of a culture significantly fitted the model, i.e. reached the stationary phase (carrying capacity), where outcome of interspecific competition is mostly pronounced, was defined as the first of 3 days in the stationary phase, after which the cultures were terminated. Cell abundance and cell size were used to calculate total biovolume as a measure of total biomass.

At the end of the experiment all experimental cultures were sampled for community composition, DIC, TA, and total particulate carbon (TPC). Community composition was determined using the Phenom G2 pure desktop SEM. Samples were prepared and analysed as described above. DIC samples were taken with a peristaltic pump and filtered through single-use syringe filters (0.2 µm, Minisart RC25, Sartorius, Göttingen, Germany) into 10 mL glass vials that were immediately sealed with butyl rubber-septa (Hansen et al. 2013). Subsequent analysis was carried out with a gas chromatography system (SRI-8610, Torrance, USA). TA samples were filtered through GF/F filters (Whatman, Dassel, Germany) and analysed using a Titrino plus 848 (Metrohm, Filderstadt, Germany). The remaining carbonate parameters were calculated using CO2SYS (Pierrot et al. 2006) and the constants supplied by Hansson (1973) and Mehrbach et al.(1973), that were refitted by Dickson & Millero (1987) and the KSO_4 dissociation constant from Dickson (1990). TPC samples were taken on pre-combusted GF/F filters (Whatman, Dassel, Germany). Before analysis, filters were dried at 60 °C, folded, packed in tin cups, and subsequently analysed using an organic

elemental analyser with a thermal conductivity detector (FlashEA® 1112, Thermo Scientific, Soeberg, Denmark).

Statistical analyses

Prior to statistical analyses, data were tested for normality and homogeneity of variances. If data were not normally distributed or variances were not homogeneous, data were log transformed. Addressing hypothesis (i), we tested the effect of species identity and elevated CO_2 and their interaction on the performance of individual species during overwintering by calculating a 2-way analysis of variance (ANOVA) among the monocultures. Student's *t*-tests were performed in terms of final total biovolume to analyse if the community assembly using individuals from the overwintered monocultures harboured no effect itself (*Mix-low* vs. *Control*/*Mix-high* vs. *Stressed*); addressing hypothesis (iii), to disentangle the pure ecological effect of elevated CO_2 (*Community* vs. *Control*) from the combined physiological and ecological effect (*Control* vs.*Stressed*); and addressing hypothesis (iv), to test for potential correlated responses to selection (*History* vs. *Stressed*). *P* values were adjusted by sequential Bonferroni corrections (Holm 1979, Rice 1989).

The consistency of the pattern we found in total biovolume with the TPC pattern was confirmed by a positive Pearson correlation coefficient ($r = 0.95$, $P < 0.001$, $N = 24$).

Results and discussion

Effects of elevated CO_2 during overwintering

Phytoplankton seasonal succession is not a start from zero abundance and the widespread lack of attention to the role of overwintering conditions for phytoplankton communities at the onset of a spring bloom might miss important mechanisms. In the present study, the performance among individual species during overwintering differed across both CO_2 levels (Fig. 2; ANOVA, significant main effect of species identity: $F_{2,18} = 25.0$, $P < 0.001$). *Gephyrocapsa muellerae* exhibited most cell divisions (≈ 5.4) during overwintering, followed by *Emiliania huxleyi* (≈ 4.3), and lastly *Calcidiscus quadriperforatus* (≈ 2.6) (Fig. 2). The lower growth of *C. quadriperforatus* is in line with previous studies showing that growth rates of phytoplankton species decrease with increasing cell size (Chrisholm 1992, Tang 1995). Depending on species identity the effect of elevated CO_2 on the performance of individual species during overwintering varied (Fig. 2; ANOVA, significant interaction species identity x CO_2: $F_{2,18} = 3,6$, $P < 0.05$) (accepting hypothesis i). *G. muellerae* was favoured under *Acidified* conditions, whereas *E. huxleyi* performed better under *Non-Acidified* conditions. *C. quadriperforatus* showed no response to elevated CO_2. This aforementioned variability among species in response to elevated CO_2 is in accordance with previous studies

(Riebesell et al. 2000, Langer et al. 2006). Looking at the intraspecific level, existing literature further reveals even strain-specific responses to changing seawater chemistry (Langer et al. 2009). Overwintering strategies of phytoplankton generally include overwintering in the active phase or in a resting stage. A distinctive feature in the biology of coccolithophores however, is that resting stages are seemingly absent (Billard & Inouye 2004).

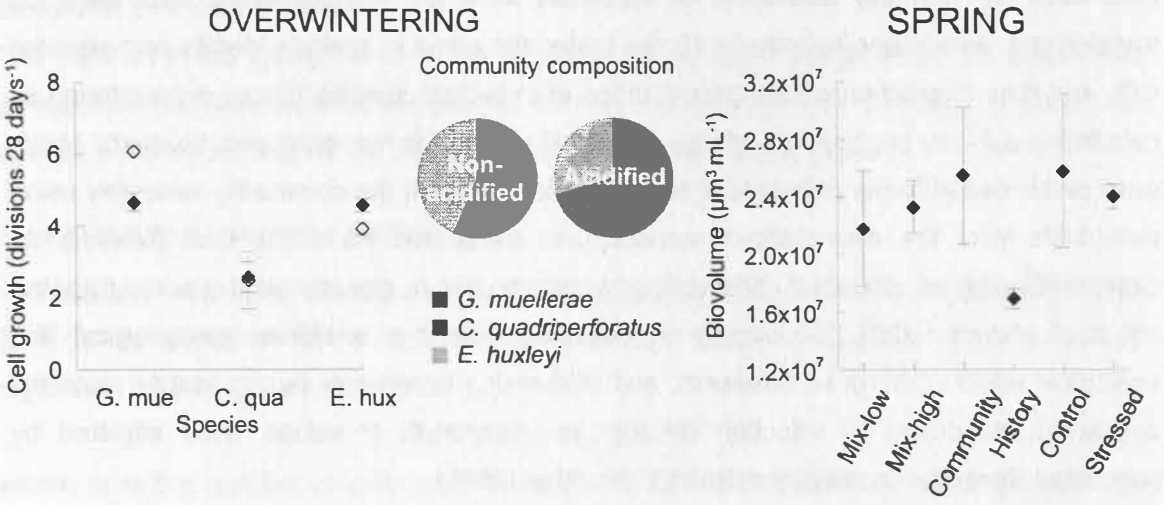

Fig. II-2. Cell growth during the overwintering phase of the three different species in monocultures for both CO_2 levels (Non-acidified = closed diamonds; Acidified = open diamonds) (left). Community composition of the overwintered polycultures for both CO_2 levels (middle). Final total biovolume in the six different treatments of the spring phase (right).

Compared to ecological effects, physiological effects occur relatively fast (Collins & Gardner 2009). Physiological short-term acclimation is the process in which an individual organism adjusts to environmental change, such as elevated CO_2, allowing it to maintain performance across a range of environmental conditions. Organisms can adjust their morphological, behavioural, physical, and/or biochemical traits in response to changes in their environment. Phenotypic plasticity is the ability of an organism to change its phenotype in response to changes in the environment. A concept that places phenotypic plasticity in the context of a genotype-specific response is the norm of reaction. A norm of reaction is an array of phenotypes that will be developed by a genotype over a range of environments.

Owing to the above described physiological effects on individual species, elevated CO_2 altered the community composition during overwintering (accepting hypothesis ii). Analyses of the relative biovolume of each species after the overwintering phase showed that in both CO_2 levels *Gephyrocapsa muellerae* was the dominant species (Fig. 2). Its contribution to total biovolume under *Acidified* conditions (69%) however, was higher compared to that under *Non-acidified* conditions (56%) (Fig. 2). The contribution of *Emiliania huxleyi* to total biovolume was 30 and 43% under *Acidified* and *Non-Acidified* conditions, respectively (Fig. 2). *Calcidiscus quadriperforatus* contributed in both CO_2 levels ≈ 1% (Fig. 2).

Physiological vs. ecological effect of elevated CO_2

First of all, the community assembly using individuals from the overwintered monocultures harboured no effect itself (Student's *t*-tests: *Mix-low* vs. *Control* and *Mix-high* vs. *Stressed*: n. s.).

The *Stressed* treatment included both the direct physiological effect of elevated CO_2 on individual species during overwintering and the indirect ecological effect of altered initial community composition at the onset of the spring phase. Comparing the *Stressed* treatment with the *Control* treatment however, showed that elevated CO_2 had no effect at all (Student's *t*-test: *Stressed* vs. *Control*: n. s.).

The *Community* treatment on the other hand, was designed to include only the indirect ecological effect of altered initial community composition at the onset of the spring phase. This was realised by assembling communities in the species ratio observed in the polycultures after overwintering under *Acidified* conditions using individuals from *Non-acidified* overwintered monocultures and inoculating them into *Non-acidified* conditions. Comparing the *Community* treatment with the *Control* treatment revealed that initial community composition had no effect on biovolume (Student's *t*-test: *Community* vs. *Control*: n. s.). The subtraction of the ecological effect of initial community composition from the combined ecological and physiological effect would have given the pure physiological effect of elevated CO_2. Since we found neither a significant combined effect nor a significant pure ecological effect, we conclude that there was also no physiological effect of elevated CO_2 on biovolume. The absence of an effect of initial community composition on community functioning is in line with previous studies using the same species as a model system (Eggers & Matthiessen 2013). Here, priority effects affected community structure but did not translate into altered community functioning. In the present study, all treatments were highly dominated by *Emiliania huxleyi* at the end of the experiment despite *Gephyrocapsa muellerae* being the dominant species at the onset of the spring phase. The contribution of *E. huxleyi* to final biovolume was > 95% in those communities that started the spring phase in the species ratio observed in the polycultures after overwintering under *Non-acidified* conditions and > 85% in those communities that started the spring phase in the species ratio observed in the polycultures after overwintering under *Acidified* conditions. This difference of ≈ 10% reflects that the contribution of *G. muellerae* to total biovolume was higher in the overwintered polycultures under *Acidified* conditions. We conclude that the onset of the spring phase along with higher temperature and increased light intensity favoured the superior growth of *E. huxleyi* to such an extent that the initial numerical advantage of *G. muellerae* vanished.

Potential selection effect of elevated CO_2

The *History* treatment examined the correlated responses to ecological selection (species sorting) and potentially evolutionary genotypic selection as performance of communities selected at elevated CO_2 during overwintering when exposed back to *Non-acidified* conditions in the species ratio observed in the polycultures after overwintering under *Non-acidified* conditions. Comparing the *History* treatment with the *Stressed* treatment revealed that the total biovolume was significantly lower in the *History* treatment (Student's *t*-tests: *History* vs. *Stressed*: $t = -6.36$, $P < 0.001$, $P_{adjusted} = 0.01$, N = 8). Previous studies likewise found reduced growth when exposing high-CO_2-selected populations of *Chlamydomonas reinhardtii* back to the ambient environment, indicating a degeneration of carbon-concentrating mechanisms by conditionally neutral mutations (Collins & Bell 2004). The absence of an ecological effect of elevated CO_2, hints towards the possibility that instead we rather found a genotypic selection effect among differentiated strains within species. It is now established that phytoplankton possess standing genetic variation in the face of multiple stressors including elevated CO_2 and warming (reviewed in Reusch & Boyd 2013).

However, the most powerful direct test, which is lacking in the present design, would be the comparison of communities selected at *Acidified* conditions with those grown under *Non-acidified* conditions both exposed back to *Acidified* conditions (Lohbeck et al. 2012). Ideally, one would need to have as additional treatments monocultures exposed vs. not exposed to acidification which would both be used to assemble all possible combinations of communities for an additional decomposition of evolutionary and ecological effects.

Outlook

The present study was based on only one functional group–coccolithophores. Because phytoplankton functioning depends on trait composition, it remains a major challenge to understand how phytoplankton communities harboring all their functional variability will reorganize in response to climate change in order to predict the impact on future oceans' ecosystems. Hence, in order to further unravel the interplay between physiological, ecological, and evolutionary effects of elevated CO_2 on ecosystem functioning, it is necessary to include a variety of functional groups in future experiments.

Acknowledgements

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CHAPTER III

Community composition has greater impact on the functioning of marine phytoplankton communities than ocean acidification

Abstract

Ecosystem functioning is simultaneously affected by changes in community composition and environmental change such as increasing atmospheric carbon dioxide (CO_2) and subsequent ocean acidification. However, it largely remains uncertain how the effects of these factors compare to each other. Addressing this question, we experimentally tested the hypothesis that initial community composition and elevated CO2 are equally important to the regulation of phytoplankton biomass. We full-factorially exposed three compositionally different marine phytoplankton communities to two different CO_2 levels and examined the effects and relative importance (ω^2) of the two factors and their interaction on phytoplankton biomass at bloom peak. The results showed that initial community composition had a significantly greater impact than elevated CO^2 on phytoplankton biomass, which varied largely among communities. We suggest that the different initial ratios between cyanobacteria, diatoms, and dinoflagellates might be the key for the varying competitive and thus functional outcome among communities. Furthermore, the results showed that depending on initial community composition elevated CO^2 selected for larger sized diatoms, which led to increased total phytoplankton biomass. Our study highlights the relevance of initial community composition, which strongly drives the functional outcome, when assessing impacts of climate change on ecosystem functioning. In particular, the increase in phytoplankton biomass driven by the gain of larger sized diatoms in response to elevated CO^2 potentially has strong implications for nutrient cycling and carbon export in future oceans.

Introduction

Since the industrial revolution, human activities have been altering the composition of biological communities at all scales, from local to global, at an unprecedented and accelerating rate (Lotze et al. 2006). Ecological experiments, observations, and theoretical developments show that ecosystem functioning depends greatly on biodiversity in terms of the functional characteristics of organisms present in the ecosystem and the distribution and abundance of those organisms over space and time (Kinzig et al. 2002, Loreau et al. 2002). Ecosystem functioning encompasses the properties, goods, and services of ecosystems (Christensen et al. 1996), although here we use the term ecosystem functioning as synonymous with ecosystem properties. These generally include both state variables of compartments (e.g., pools of materials such as carbon or organic matter) and rates of processes (e.g., fluxes of materials and energy between compartments). Evidence is mounting that the effects of biodiversity loss or changes in community composition strongly alter ecosystem properties and the goods and services they provide to humanity (Hooper 2005), and thus have been the focus of much ecological research (Stachowicz et al. 2007, Hillebrand et al. 2008, Hillebrand & Matthiessen 2009, Cardinale et al. 2012). To date it remains largely uncertain, however, how biodiversity effects on ecosystem functioning compare to direct effects of other forms of environmental change, such as increasing atmospheric carbon dioxide (CO_2) and subsequent ocean acidification and climate warming. A lack of factorial experiments in the past, led Hooper et al. (2012) to take a first comprehensive effort to directly compare the effects of changes in biodiversity—in this case, loss of species richness—to other environmental changes by using a combined meta-analytic approach. They found that loss of species richness affects ecosystem functions as much as climate change, pollution, and other major forms of environmental stress.

As stated above, biodiversity encompasses not only species richness but also relative abundance and composition. Profound changes in ecosystem functioning can be expected when altered initial species composition at the start of a growing season leads to changed competitive outcome. Fukami et al. (2010), for instance, found, using wood-decaying fungi as a model system, that direct manipulation of early immigration history resulted in threefold differences in fungal species richness and composition and, as a consequence, differences of the same magnitude in the rate of decomposition and carbon release from the wood. Another study, using a microbial system, revealed that stability of net ecosystem denitrification in the face of salinity stress was strongly influenced by initial evenness of the community (Wittebolle et al. 2009).

Phytoplankton harbour an extremely diverse, polyphyletic group of microscopic photosynthetic protists (algae) and cyanobacteria. Due to their small size, short generation

times, and large population numbers phytoplankton are an excellent model system to address fundamental ecological questions. Phytoplankton contribute approximately half of the world's primary production (Falkowski 2012), giving them an important role as the basis of the pelagic food web. Moreover, Laws et al. (2000) showed that acting as a biological carbon pump, phytoplankton and other organisms in the sunlit layer export up to 15% of the organic material produced each year to the deep sea, where about 0.1% of it gets buried in the sediment. Major taxonomic groups of eukaryotic phytoplankton can be classified into distinct functional groups (Iglesias-Rodríguez et al. 2002) with unique biogeochemical signatures. Phytoplankton community composition profoundly affects the biogeochemical cycling of many elements, such as carbon, nitrogen, and phosphorus, because major functional groups have different requirements and modes of acquisition of these elements (Falkowski et al. 2004). Important parameters of nutrient uptake scale linearly with cell surface area, because the number of uptake locations increases with increasing cell surface area (Aksnes & Egge 1991). Many cyanobacteria are able to fix atmospheric nitrogen and increase nitrogen availability in the water column (Howarth et al. 1988). Diatoms convert soluble silicic acid to solid hydrated amorphous opal. They exhibit high maximum growth rates and appear to be good competitors for inorganic nutrients such as nitrogen (Litchman et al. 2007). Diatoms tend to dominate phytoplankton communities except when silicon is scarce (Egge & Aksnes 1992). Coccolithophores in turn, such as Emiliania huxleyi, convert dissolved inorganic carbon and calcium to solid-phase calcium carbonate (CaCO3). They can dominate blooms when diatoms are excluded from competition, for instance, when other nutrients are still abundant but dissolved silicate has been exhausted. An important trait that aids in nutrient and sometimes carbon acquisition in many groups of phytoplankton is mixotrophy (presence of heterotrophic and autotrophic modes of nutrition); usually those groups are poor competitors for inorganic nutrients, such as dinoflagellates (Litchman et al. 2007). These functional traits, among others, exhibit trade-offs that define contrasting ecological strategies in major functional groups and thus contribute to phytoplankton community composition and subsequent ecosystem functioning.

Sensitivity against ocean acidification has been found in many marine organisms, including major phytoplankton groups (Rost et al. 2008, Doney et al. 2009). Ocean acidification is the consequence of increasing atmospheric carbon dioxide (CO_2), which dissolves in seawater and subsequently increases seawater acidity and decreases carbonate ion concentration. Due to the wide variety of processes affected, responses to ocean acidification vary broadly across and even within taxa. Most of the elevated CO_2 response studies on phytoplankton, whether for calcification (Riebesell et al. 2000, Langer et al. 2006, Barcelos e Ramos et al. 2010), photosynthesis (Gattuso et al. 1999, Rost & Riebesell 2004), or other physiological parameters, have been primarily focused on single organisms. Based on a meta-analytic

approach, Kroeker et al. (2010) revealed that calcifying organisms generally exhibit larger negative responses to ocean acidification than non-calcifying organisms across numerous response variables. Studies testing effects of elevated CO_2 on whole phytoplankton communities are comparatively rare. The few available examples from different regions show that ocean acidification might lead to a shift in community composition (Tortell et al. 2008, Schulz et al. 2013), leaving the question open of how ecosystem functioning might be affected.

We used marine phytoplankton communities to experimentally tackle the question of how differences in initial community composition affect biomass build-up of a bloom compared to the direct effects of ocean acidification. Specifically, we hypothesized that (i) size and direction of the elevated CO_2 effect depend on initial community composition; and (ii) both factors, initial community composition and elevated CO_2, are equally important to the regulation of phytoplankton biomass.

Methods

Experimental design

The experiment took place in April 2012 on Terceira Island (Azores, North Atlantic). Three compositionally different phytoplankton communities were full-factorially exposed to two different CO_2 levels. Each treatment combination (initial community composition x CO_2) was quadruplicated, resulting in 24 experimental units comprising 5 L plastic bottles that were randomly deployed around a pier at a water depth of approximately 2 m.

At the onset of the experiment, nutrients were added to 120 L of sterile filtered seawater (0.2 µm) with a salinity of 36 PSU to achieve concentrations of 6 µmol L-1 silicate (Si), 8 µmol L-1 nitrate (N), and 0.5 µmol L^{-1} phosphate (P). This reflects a natural pre-bloom nutrient regime for this region (ICES Oceanographic Database 2013). Vitamin and trace metal concentrations corresponded to one-tenth of a f / 2 – medium (Guillard 1975). After nutrient addition the seawater was transferred into six 20 L polypropylene bottles.

Initial CO_2 was manipulated by adding $NaHCO_3$ and HCl following Schulz et al. (2009), which increased dissolved inorganic carbon (DIC) at constant total alkalinity (TA). The two levels of initial CO_2 corresponded to a CO_2 partial pressure (pCO_2) of 382 ± 13 µatm ('Non-acidified', pHT ≈ 8.1) and 907 ± 44 µatm ('Acidified', pHT ≈ 7.7), respectively, whereas TA was 2389 ± 7 µmol kg SW^{-1} in both CO_2 levels.

The three compositionally different phytoplankton communities were obtained by collecting seawater from the integrated upper 10 m of the water column at three different sites offshore from Terceira Island. The three sites varied in their geographical characteristics, such as current exposure and distance to the shore (ds) (Site 1: 38°38'N 27°04'W, ds ≈ 400 m; Site 2:

38°39'N 27°15'W, ds ≈ 500 m; Site 3: 38°37'N 27°15'W, ds ≈ 4000 m). In order to avoid grazing pressure by large zooplankton, the seawater was immediately filtered through a 200 µm pore size mesh. Afterwards, 15 L of seawater per site was distributed among ten 1.5 L bottles. In order to concentrate the phytoplankton in the bottle necks by sedimentation, each bottle was placed upside down in a climate-controlled cabinet. Light and temperature conditions in the cabinet were similar to natural conditions. After 24 hours 200 mL of water was collected from the bottom of each bottle through a valve and collected in a beaker. Relative fluorescence of the pooled concentrated phytoplankton of each site was measured with the 10AU Field and Laboratory Fluorometer by Turner Designs (Sunnyvale, USA). In order to avoid potential density effects and warrant equal initial biomass among the three different communities (C1, C2, and C3 originated from Site 1, Site 2, and Site 3, respectively), the particular volume necessary for inoculation was calculated from the relative fluorescence data. The primary objective of using the relative fluorescence data was not to define the absolute initial phytoplankton biomass but to ensure that the initial phytoplankton biomass in all three communities was equal relative to each other. Each 20 L treatment combination (initial community composition x CO_2) was subsequently inoculated with phytoplankton from its respective site of origin. After gentle mixing, the inoculated seawater was transferred into the actual experimental units. In order to minimize the potential effect of the seawater that comes along with the inocula on the prepared media, we ensured that the volume of the inocula did not exceed 5% of the total volume in each experimental unit. The initial phytoplankton biomass corresponded to 8.11 µg C L^{-1} ± 1.35 µg C L^{-1} in each treatment combination, which was less than 1% of the final phytoplankton biomass. During the experiment, aggregation and sedimentation was limited by carefully rotating the bottles two times a day (ten rotations each time) in the shade to prevent prolonged exposure to high light conditions. To ensure that all communities were sampled in the same growth phase the exact duration of each culture was determined by a sigmoidal growth model (see below). Depending on the culture duration, the experimental units ran for nine to ten days.

Sampling and response variables

Each experimental unit was sampled daily (15 mL) to measure relative fluorescence and assure that all communities were sampled in the same growth phase. The total volume removed over the course of the experiment was about 3% in each experimental unit. The decision to terminate a culture was based on the statistically significant fit to a sigmoidal growth model:

$$ft = a \times \{1+[(a - b)/b] \times e^{(-\mu \times t)}\}-1 \qquad \text{(III-1)}$$

where *ft* indicates the relative fluorescence after *t* days, *a* the maximum relative fluorescence (carrying capacity), *b* the start relative fluorescence, and µ the growth rate. The first day that

the growth curve of a culture significantly fitted the model, i.e. reached the stationary phase (carrying capacity), was defined as the first of three days in the stationary phase, after which the cultures were terminated. All cultures were then directly sampled for the following parameters: dissolved inorganic carbon (DIC), total alkalinity (TA), pH_T, dissolved inorganic nutrients (Si, N, P), total particulate carbon and nitrogen (TPC, TPN), particulate organic carbon and nitrogen (POC, PON), community composition, and phytoplankton biomass.

DIC samples were taken with a peristaltic pump and filtered through single-use syringe filters (0.2 µm, Minisart RC25, Sartorius, Goettingen, Germany) into 10 mL glass vials that were immediately sealed with butyl rubber-septa (Hansen et al. 2013). Subsequent analysis was carried out with a gas chromatographic system (SRI-8610, Torrance, USA). TA samples were filtered through GF/F filters (Whatman, Dassel, Germany) and analysed using the Titrino plus 848 (Metrohm, Filderstadt, Germany). The remaining carbonate parameters were calculated using CO2SYS (Pierrot et al. 2006) and the constants supplied by Hansson (1973) and Mehrbach et al. (1973), that were refitted by Dickson & Millero (1987) and the KSO4 dissociation constant from Dickson (1990) (Appendix A). Samples for dissolved inorganic Si, N, and P were filtered through cellulose acetate membrane filters (0.2 µm) and measured by continuous flow analyses (CFA) following Hansen & Koroleff (1999) using a SKALAR SANPLUS auto-analyzer system (Breda, The Netherlands). TPC, TPN, POC, and PON samples were taken on pre-combusted and hydrochloric acid treated GF/F filters (Whatman, Dassel, Germany). For POC samples, the particulate inorganic carbon was removed from filters by exposing them to fuming hydrochloric acid for 12 hours. Before analysis, filters were dried at 60°C, folded, packed in tin cups, and consequently analysed using an organic elemental analyser with a thermal conductivity detector (Flash 2000, Thermo Scientific, Soeborg, Denmark). Particulate inorganic carbon (PIC) was calculated by subtracting POC from TPC. Since the final portion of calcifying phytoplankton, more precisely coccolithophores, was low in all communities, POC was hardly distinguishable from TPC and PIC was consequently negligible. We therefore show only the results of TPC.

Three different phytoplankton samples were taken in order to assess initial as well as final community composition and phytoplankton biomass with a focus on functional groups. Samples for initial community composition and biomass were obtained from the inocula. For species > 5 µm cell size samples were fixed with Lugol's iodine and identified as well as counted with an inverted microscope (Axio Observer.A1, Zeiss) following Utermöhl (1958). Since identification of *Chaetoceros* to the species level was highly difficult in the final samples, *Chaetoceros* spp. were grouped in three size classes, representing an average cell biovolume of 85 (*I*), 431 (*II*), and 831 μm^3 (*III*), respectively. Species < 5 µm cell size were analysed by flow cytometry (BD FACSCalibur, Heidelberg, Germany). Coccolithophores were determined by scanning electron microscopy using a Phenom G2 pure desktop SEM

(Eindhoven, The Netherlands). SEM samples were filtered on Nuclepore track-etched polycarbonate membranes (0.8 µm, Whatman, Dassel, Germany). Phytoplankton biomass was calculated after approximation to geometric models (Hillebrand et al. 1999), complemented by data from the HELCOM phytoplankton check list (Olenina et al. 2006), and converted into carbon content as described by Menden-Deuer & Lessard (2000). The consistency of the calculated carbon (referred to in this manuscript as phytoplankton biomass) with the measured TPC was confirmed by a positive Pearson correlation ($r = 0.68$, slope = 0.77, $p < 0.01$, $N = 24$). Data were used to determine evenness and richness of each community as well as the relative contribution of each phytoplankton group. This was done applying the highest possible taxonomic resolution. Since diatoms were dominating the final communities, initial evenness and richness was additionally determined for diatoms only.

Data analysis

Bray-Curtis dissimilarities (Bray & Curtis 1957) were used to quantify the initial compositional dissimilarities among the three communities. Final data were tested for normality and homogeneity of variances before statistical analyses were undertaken. If data were not normally distributed or variances were not homogeneous, data were log transformed. Addressing our hypotheses, we tested the effects of initial community composition (com), CO_2, and their interaction on TPC, phytoplankton biomass, PIC, evenness, dissolved inorganic N, P, and Si by calculating a two-way ANOVA. Effect size was calculated as omega squared ($\omega 2 = SS_{treatment} - df_{treatment} * MS_{error}) / (SS_{total} + MS_{error})$) in order to determine the relative importance of each of the factors (Hughes & Stachowicz 2009). In order to identify significant differences among the three communities as well as between the two CO2 levels within each of the three communities, Tukey's HSD (honestly significant difference) post-hoc tests were used. Using SIMPER analysis (Clarke 1993) implying Bray-Curtis dissimilarity, the groups primarily driving the discrimination in biomass were identified between those communities differing from each other in terms of TPC and phytoplankton biomass and between CO_2 levels within those communities where elevated CO_2 led to higher TPC and phytoplankton biomass.

Results

Initial community composition

The initial compositional dissimilarity was 47% between C1 and C2, 48% between C1 and C3, and 42% between C2 and C3. Initial evenness was similar among the three different communities, representing 0.67, 0.66, and 0.65, for C1, C2, and C3, respectively. Initial richness was also similar, with 47 species in C1 and 52 species in both C2 and C3. Despite

this high initial similarity among the three communities, however, they clearly differed in the relative composition of each group. C1 was dominated by diatoms (48%), followed by cyanobacteria (32%) and dinoflagellates (16%) (Fig. 1). Nanoflagellates, coccolithophores, ciliates, and picoplankton contributed less than 5%. In C2 diatoms were less abundant (28%) (Fig. 1). Instead, C2 was dominated by dinoflagellates (37%). The portion of cyanobacteria was half that of C1 (16%), and in C2, coccolithophores and ciliates accounted for 9% and 7%, respectively (Fig. 1). Nanoflagellates and picoplankton were both below 5% (Fig. 1). C3 was dominated by dinoflagellates (29%) and cyanobacteria (26%) (Fig. 1) and held the lowest portion of diatoms (8%) but the highest share of coccolithophores (19%) (Fig. 1). The portion of ciliates (11%) and nanoflagellates (6%) was greater in C3 than in C1 and C2, whereas the contribution of picoplankton was below 5% (Fig. 1). Initial evenness of only diatoms ranged between 0.74 and 0.71. Species richness of diatoms initially was 18 for both C1 and C2. A lower initial species richness of diatoms, 12, was found in C3.

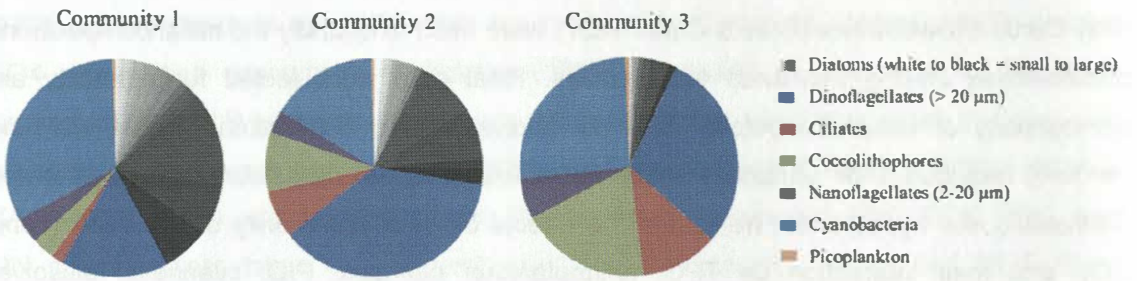

Fig. III-1. Initial composition of the three communities expressed as relative biomass of each group. The different diatom species are shown using a white to black spectrum to indicate size (smaller to larger).

Contribution of community composition vs. CO_2

Initial community composition, CO_2, and the interaction of these two factors significantly affected TPC (Table 1). Calculation of the effect sizes revealed that initial community composition explained 77% of the variance, whereas CO_2 and the interaction of community composition and CO_2 accounted for only 5% and 4%, respectively (Table 1). TPC was on average a quarter higher in C1 and C3 compared to C2 (Tukey HSD: $p < 0.001$) (Fig. 2a). TPC values between C1 and C3 were not significantly different from each other (Tukey HSD: $p > 0.05$) (Fig. 2a). Elevated CO_2 significantly increased TPC by about 22% in C1 (Tukey HSD: $p < 0.05$) (Fig. 2a). Although the effect of elevated CO_2 was not significant in C2 and C3 (Tukey HSD: $p > 0.05$), in C2 TPC levels tended to increase by about 12% (Fig. 2a).

Phytoplankton biomass was significantly affected by initial community composition, CO_2, and the interaction of the two (Table 1). Initial community composition explained 61% of the variance, whereas CO_2 and the interaction of initial community composition and CO_2 accounted for 5% and 14%, respectively (Table 1). Phytoplankton biomass was on average a quarter higher in C1 and C3 compared to C2 (Tukey HSD: $p < 0.001$) (Fig. 2b).

Phytoplankton biomass between C1 and C3 did not significantly differ (Tukey HSD: $p > 0.05$) (Fig. 2b). Elevated CO_2 significantly increased phytoplankton biomass by about 24% in C2 (Tukey HSD: $p < 0.01$) (Fig. 2b). Although the effect of elevated CO_2 was not significant in C1 and C3 (Tukey HSD: $p > 0.05$), in C1 phytoplankton biomass tended to increase by about 14% and in C3 by about 10% (Fig. 2b).

Table III-1. ANOVA results explaining the effects of initial community composition (com) and CO_2 and these factors' combined interaction on total particulate carbon (TPC), phytoplankton biomass (bm), the C:N ratio (C:N), evenness, dissolved inorganic nitrogen (N), phosphorous (P), and silicon (Si). Omega squared (ω^2) indicates the effect size of the contributing factors. Values in bold are significant at $p < 0.05$.

		Whole model				Contributing factors			
Variable	Factor	df	R^2	F	*p*-value	df	F	*p*-value	ω^2
log TPC		5, 18	0.86	30.13	**< 0.001**				
	com					2, 18	66.6	**< 0.001**	0.77
	CO_2					1, 18	8.7	**< 0.01**	0.05
	com x CO_2					2, 18	4.4	**< 0.05**	0.04
log bm		5, 18	0.81	20.51	**< 0.001**				
	com					2, 18	38.3	**< 0.001**	0.61
	CO_2					1, 18	7.3	**< 0.05**	0.05
	com x CO_2					2, 18	9.4	**< 0.01**	0.14
log C:N		5, 18	0.88	34.13	**< 0.001**				
	com					2, 18	80.77	**< 0.001**	0.84
	CO_2					1, 18	8.66	**< 0.01**	0.04
	com x CO_2					2, 18	0.23	0.80	
Evenness		5, 18	0.76	15.54	**< 0.001**				
	com					2, 18	35.98	**< 0.001**	0.72
	CO_2					1, 18	0.82	0.38	
	com x CO_2					2, 18	2.46	0.11	
N		5, 18	0.36	3.63	**< 0.05**				
	com					2, 18	8.30	**< 0.01**	0.39
	CO_2					1, 18	0.45	0.51	
	com x CO_2					2, 18	0.56	0.58	
P		5, 18	-0.03	0.84	0.54				
	com					2, 18	1.10	0.35	
	CO_2					1, 18	0.39	0.54	
	com x CO_2					2, 18	0.81	0.46	
Si		5, 18	0.85	28.02	**< 0.001**				
	com					2, 18	61.67	**< 0.001**	0.77
	CO_2					1, 18	5.13	**< 0.05**	0.03
	com x CO_2					2, 18	5.82	**< 0.05**	0.06

Initial community composition and CO_2 but not the interaction of the two factors significantly affected the POC:PON ratio (Table 1). For the POC:PON ratio, 84% of the variance was explained by initial community composition, whereas CO_2 accounted for 4% (Table 1). In both C1 and C3 the POC:PON ratio was on average twice as high compared to C2 (Tukey

HSD: $p < 0.001$) (Fig. 2c). The POC:PON ratio did not significantly differ between C1 and C3 (Tukey HSD: $p > 0.05$) (Fig. 2c). Although the ANOVA revealed a significant main effect of CO_2 on the POC:PON ratio (Table 1), significant differences between the two CO_2 levels could not be detected in any of the communities when compared by Tukey HSD ($p > 0.05$). However, with elevated CO2 the POC:PON ratio tended to increase on average about 11% (Fig. 2c).

Final evenness was significantly driven by initial community composition, which explained 72% of the variance, with the highest final evenness in C2 and the lowest in C3 (Tukey HSD: $p < 0.001$) (Table 1; Fig. 2d).

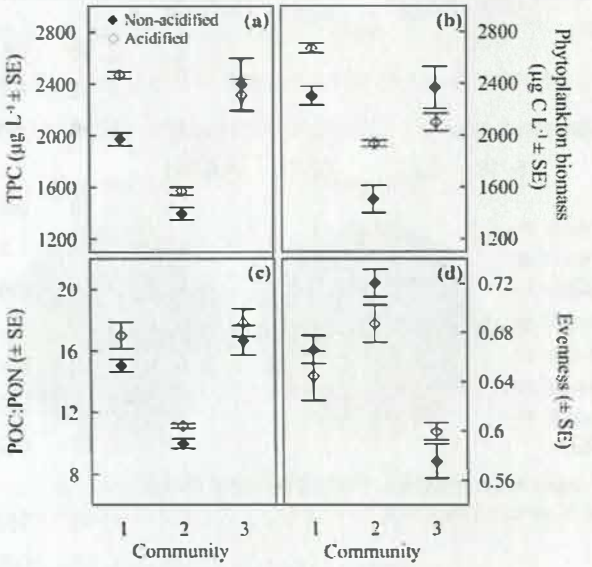

Fig. III-2. Total particulate carbon (TPC), phytoplankton biomass, the ratio of particulate organic carbon to particulate organic nitrogen (POC:PON), and evenness in the three different communities for both CO_2 levels (Non-acidified = closed diamonds; Acidified = open diamonds).

Primarily phytoplankton groups driving differences in final biomass

In general, at the end of the experiment all communities were dominated by diatoms, particularly by the different size classes of *Chaetoceros* and by *Thalassiosira constricta*. The identities of the species but not their contributions were similar in all communities.

The compositional dissimilarity at the end of the experiment between C1 and C2 as well as between C2 and C3 was 37% (Table 2), with 70% of this dissimilarity explained by four groups (Table 3). TPC in C1 was enhanced compared to C2 because biomass of large and medium-sized *Chaetoceros* (*II* and *III*) was higher (Table 2; Fig. 3). Additionally, biomass of *Thalassiosira constricta* was higher in C1 compared to C2, whereas biomass of cyanobacteria was lower (Table 2; Fig. 3). In C3 TPC was enhanced compared to C2 because biomass of small and medium-sized *Chaetoceros* (*I* and *II*) as well as cyanobacteria was higher (Table 2; Fig. 3). Contrary to this, *Thalassiosira constricta* showed lower biomass in C3 compared to C2 (Table 2; Fig. 3).

Table III-2. Groups that contributed > 10% to the dissimilarity between those communities different from each other in terms of TPC and phytoplankton biomass and between CO_2 levels within C1 and C2 where elevated CO_2 led to higher TPC and phytoplankton biomass, based on SIMPER analysis.

	Bray-Curtis dissimilarity	Groups	Contribution to difference (%)	Cumulative contribution to difference (%)
Between the communities				
C1 & C2	37.34	*Chaetoceros II*	26.54	26.54
		Chaetoceros III	19.97	46.51
		Thalassiosira constricta	12.75	59.26
		Cyanobacteria	10.90	70.16
C2 & C3	37.47	*Chaetoceros I*	25.69	25.69
		Cyanobacteria	17.06	42.74
		Chaetoceros II	14.39	57.13
		Thalassiosira constricta	12.73	69.86
Between CO_2 levels within the communities				
C1	19.79	*Chaetoceros II*	26.93	26.93
		Chaetoceros III	23.17	50.10
		Thalassiosira constricta	15.88	65.98
C2	30.57	*Thalassiosira constricta*	28.65	28.65
		Chaetoceros II	19.44	48.09
		Cyanobacteria	14.41	62.50
		Chaetoceros I	13.50	76.00

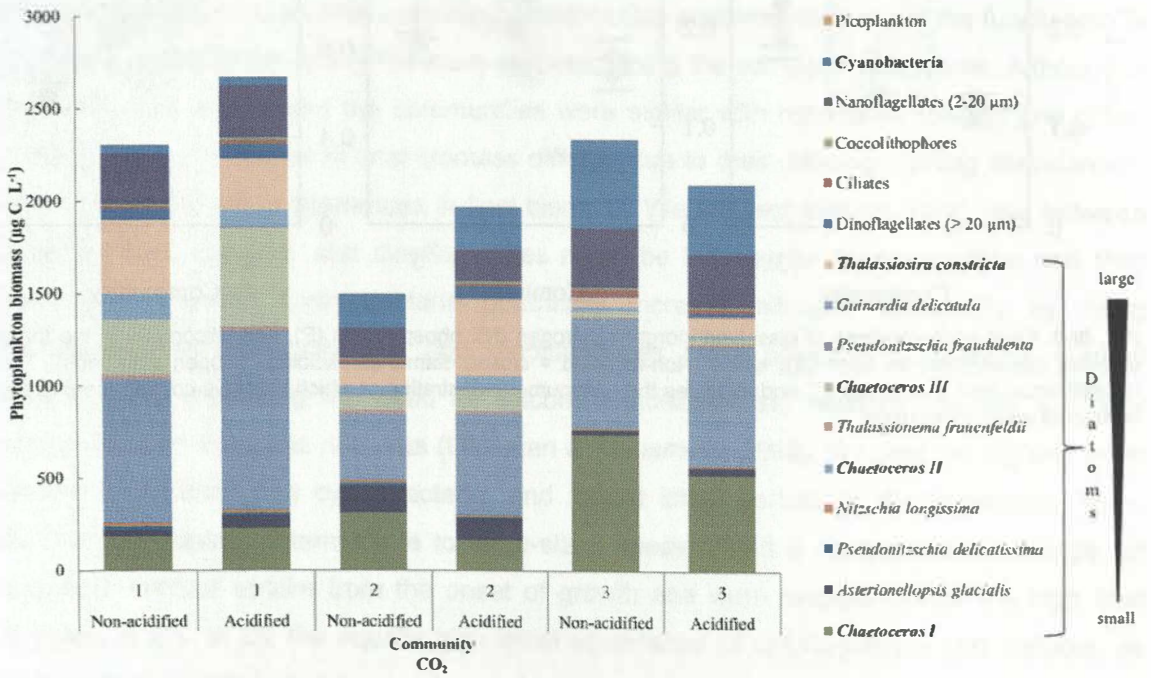

Fig. III-3. Contribution of each group to total biomass in the three different communities for both CO_2 levels. Stacking arrangement of diatoms (italic) follows cell size. Bold font indicates the groups that contributed > 10% to the dissimilarity between those communities different from each other in terms of TPC and phytoplankton biomass, and between CO_2 levels within C1 and C2 where elevated CO_2 led to higher TPC and phytoplankton biomass, based on SIMPER analysis.

Between the two CO2 levels within C1 and C2, 70% of the dissimilarity was driven by three and four groups (Table 2). In C1 TPC was enhanced with elevated CO2 because biomass of the larger-sized *Chaetoceros* (*II* and *III*) was higher (Table 2; Fig. 3). Decreasing biomass of Thalassiosira constricta with elevated CO_2 slightly counteracted this effect (Table 2; Fig. 3). In C2 TPC was enhanced with elevated CO_2 because biomass of *Thalassiosira constricta*, medium-sized *Chaetoceros II*, and cyanobacteria was higher (Table 2; Fig. 3). The lower biomass of the smaller-sized *Chaetoceros I* with elevated CO_2 slightly counteracted this effect (Table 2; Fig. 3).

Final concentration of dissolved inorganic nutrients

Dissolved inorganic phosphorous and nitrogen were depleted in all three communities in both CO_2 levels (Fig. 4a,b), however nitrogen remained higher in C3 compared to C1 and C2 (Tukey HSD: $p < 0.05$) (Table 1). Dissolved inorganic silicate was depleted in C1 and C3, however it remained significantly available in C2 (Tukey HSD: $p < 0.01$) (Table 1; Fig. 4c). The remaining concentration in C2 was significantly lower with elevated CO_2 (Tukey HSD: $p < 0.01$) (Table 1).

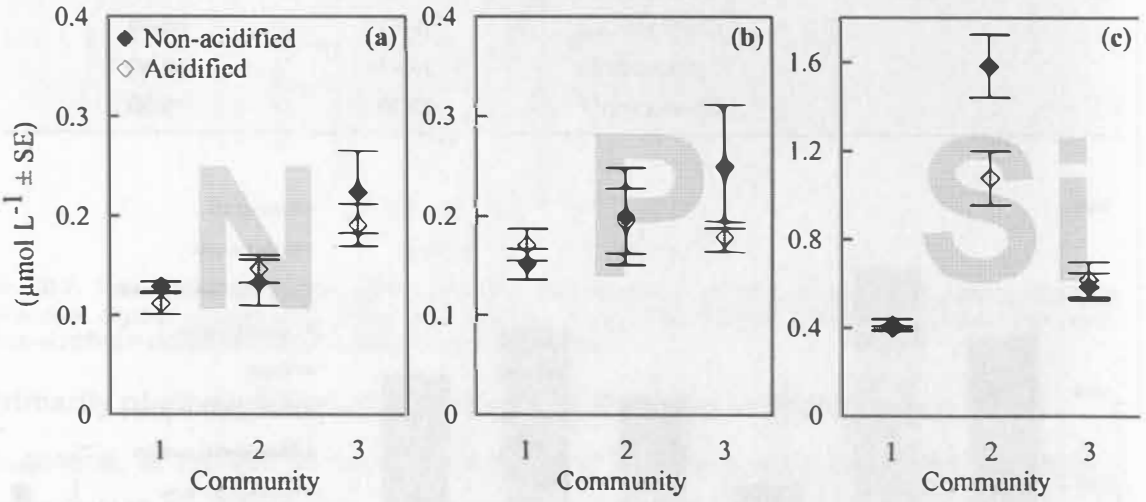

Fig. III-4. Final concentrations of dissolved inorganic nitrogen (N), phosphorous (P), and silicon (Si) in the three different communities for both CO_2 levels (Non-acidified = closed diamonds; Acidified = open diamonds). The quantification limit is 0.1 µmol L^{-1} and indicates the minimum concentration at which we have confidence that the numerical result is accurate.

Discussion

Relative effect sizes

How diversity affects ecosystem functioning compared to human mediated global change is an important unknown in ecosystem science. Hooper et al. (2012) revealed that loss of biodiversity appears to affect ecosystems as much as climate change, pollution and other major forms of environmental stress. In line with this, one of the first experimental approaches by Tilman et al. (2012) showed that decreases in grassland plant diversity influenced productivity as much as changes in resources, herbivory, or disturbance. Linking these results to the marine realm, our study aimed to experimentally assess the relative importance of initial community composition and elevated CO_2 to the functioning of a natural phytoplankton community. We found that both factors, initial community composition and elevated CO_2, are indeed significantly important for the regulation of phytoplankton biomass. However, compared to the findings of Hooper et al. (2012) and Tilman et al. (2012), and our hypothesis that both factors stack up equally against each other, initial community composition proved to have a much greater impact on phytoplankton biomass than elevated CO_2.

The importance of community composition

Our results suggest that initial community composition predominantly drove the functioning of the communities at the end of the study by determining the competitive outcome. Although at the end of the experiment the communities were similar with respect to species and group identity, their contribution to final biomass differed due to their differing starting abundances, which ultimately led to differences in final biomass. We suggest that the initial ratio between cyanobacteria, diatoms, and dinoflagellates might be the key for the competitive and thus functional outcome. Cyanobacteria potentially increase nitrogen availability by fixing atmospheric nitrogen. Diatoms and dinoflagellates have basically comparable nutrient requirements (excluding the need for silicon). Dinoflagellates, however, usually are poor competitors for inorganic nutrients (Litchman & Klausmeier 2008). C1 held the highest initial portion of diatoms and cyanobacteria, and lowest initial portion of dinoflagellates. Thus, diatoms, particularly intermediate to large-sized species, had a competitive advantage for inorganic nutrient uptake from the onset of growth and were responsible for the high final biomass in C1. In C2 the equally high initial abundance of dinoflagellates and diatoms, as well as the low initial abundance of cyanobacteria, potentially caused diatoms to be nitrogen limited before all the silicon could be exhausted. This might have prevented diatoms from reaching equally high biomass compared to C1 and C3. Initially, C3 was equally dominated

by cyanobacteria and dinoflagellates. Due to the low initial abundance of diatoms, cyanobacteria were not outcompeted in terms of phosphorous. Thus, cyanobacteria could, in addition to the in C3 competitively strong small and intermediate-sized diatoms (*Chaetoceros I* and *II*), considerably contribute to final biomass. Dinoflagellates and coccolithophores were in general competitively weak and did not significantly contribute to final biomass although they were initially abundant. Our results are in line with those of Fukami et al. (2010), who used wood-decaying fungi as a model system to prove that small differences in species immigration history during community assembly can cause large differences in ecosystem functioning. Contrary to Fukami et al. (2010), however, we did not use a temporal succession of species arriving to a patch but varied the initial relative abundance of groups. Inherently, using three independent natural communities, instead of directly manipulating biodiversity, limits the possibility for mechanistic explanation. This problem could be overcome in the future by using one known source-community in which biodiversity (i.e. the loss or distribution of given traits) is manipulated.

The final portion of calcifying phytoplankton, more precisely coccolithophores, was negligible in all communities, whereas diatoms were predominant. Diatoms typically dominate regions of upwelling, river mouths, spring blooms, and oceanographic fronts as long as there is sufficient dissolved silicate. Mesocosm experiments have confirmed that diatoms tend to dominate the phytoplankton community except when silicate is scarce (Egge & Aksnes 1992). For phytoplankton other than diatoms, like coccolithophores such as *Emiliania huxleyi*, it seems plausible that they can dominate blooms when diatoms are excluded from competition, for instance when other nutrients are still abundant but dissolved silicate has been exhausted. Aditionally, *E. huxleyi* is known to be competitively superior to other algal species under extremely phosphorus-limited conditions. Its high affinity for inorganic phosphorus and its ability to take up dissolved organic phosphates (Riegmann et al. 2000) is reflected in massive *E. huxleyi* blooms in phosphorus-depleted areas or seasons (Tyrrell & Merico 2004). Both silicate and phosphorous depletion occur towards the end of diatom dominated spring blooms. Owing to shifts in the dominant phytoplankton species caused by the given biological and environmental factors, phytoplankton blooms consequently can consist of a series of sequential blooms of different phytoplankton species.

Effects of elevated CO_2

Although inorganic carbon is mostly not considered limiting to marine primary production, its distribution in the ocean resembles that of a nutrient in some ways, such as surface depletion relative to the deep ocean and the drawdown of CO_2 during intense phytoplankton blooms (Murata et al. 2002). Consequently, transient patches of CO_2-depleted surface seawater develop which persist for several days to weeks. Physical exchange with the reservoir of DIC

in the deep sea and the reservoir of atmospheric CO_2 eventually replenishes what has been removed by the phytoplankton (Reinfelder 2011). Therefore the drawdown of CO_2 from start to end of our experiment resembles natural conditions. Furthermore, it has been shown in a mesocosm study that even when atmospheric CO_2 was kept constant, seawater CO_2 in the mesocosms largely decreased with time in both low and high CO_2 treatments. This could be clearly attributed to photosynthetic use of CO_2 (Kim et al. 2006).

Higher concentrations of CO_2 in the euphotic zone could increase the efficiency with which phytoplankton species use available limiting nutrients to fix carbon. This consequently results in phytoplankton cells with higher C:N ratios (Tortell 2000). This suggestion is in line with our results showing higher C:N ratios with elevated CO_2 in all three communities. Elevated CO_2 would also favour the growth of large cells more than small cells because larger cells are subject to greater reaction-diffusion limitation (Reinfelder 2011). The maximum uptake rate for a cell is proportional to the number of locations where encountered nutrient ions can be handled, and number of locations is considered to be related to the cell surface. The number of encountered nutrient ions in turn depends on substrate concentration causing the cell to be encounter limited at low nutrient concentrations (Aksnes & Egge 1991). In accordance to our hypothesis we found that, depending on initial community composition, elevated CO_2 selected for larger sized diatoms, especially Chaetoceros sp. and Thalassiosira constricta (Fig. 3). This required an adequate proportion of diatoms at the start of growth which was provided in C1 and C2 though less pronounced in the latter. The increase in larger sized diatoms with elevated CO_2 in C1 and C2 caused the concomitant increase in biomass. The same trend was observed in incubations of Ross Sea phytoplankton in which the occurrence of large colonial diatoms as compared to small-celled diatoms was favoured and the productivity of the assemblage increased as pCO_2 was elevated from 100 µatm to 380 µatm and to 800 µatm (Tortell et al. 2008).

Nearly all marine phytoplankton possess inorganic carbon-concentrating mechanisms (CCM) to support photosynthetic carbon fixation at the concentrations of CO_2 present in ocean surface waters (Reinfelder 2011). Active transport of inorganic carbon by CCM is thought to account for a significant portion of cellular energy expenditure (Raven 1991). Savings from CCM down-regulation are likely to be responsible for possible acclimation of oceanic phytoplankton to rising CO_2 over the next century. Allocation of energetic savings to carbon fixation is most likely to occur under conditions where growth is limited by energy gain. In this case the energy savings from down-regulation of the CCM upon increasing ambient CO_2 concentrations could, thus, increase primary production by a few percent (Hopkinson et al. 2011).

Although short-term studies of small-scale model systems like ours are apodictic simplifications, they often reveal important ecosystem processes (Srivastava et al. 2004,

Benton et al. 2007). Microcosms allow to control and manipulate particular features of a system that are difficult or impossible to control and manipulate in the field. The microcosms used in the present study were natural in a sense because they contained communities of naturally coexisting species with a shared evolutionary history. The identified functional differences between the communities underscore the limitation in using single-species studies to predict ecosystem-level effects of climate change. Certainly, our microcosms were not meant to exactly mimic a specific natural system, and thus cannot exclude any potential bottle bias, but served as a proof of concept including the role of interactions among naturally co-occurring species. Currently, we know much more about how climate will change across the globe than about how ecosystems will respond to these alterations despite the realized and expected profound effects of climate change on global biological diversity. Our study strongly suggests that initial community composition can have a much greater effect on biomass than elevated CO_2. Consequently, the importance of ocean acidification hitherto appears to be overestimated whereas the effect of community composition has been largely overlooked, although it is among the dominant drivers of changes in ecosystem functioning. In particular, our finding that the increase in phytoplankton biomass driven by the gain of larger sized diatoms in response to elevated CO_2 has potentially strong implications for nutrient cycling and carbon export in the future oceans. We propose that a major future task is to further disentangle biodiversity effects on ecosystem functioning from both direct and indirect changes in functioning due to human alterations of abiotic constraints.

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Conclusions and outlook

Predicting ecosystem responses to global change remains a major challenge in ecology. This thesis contains results of novel experimental approaches testing new hypotheses concerning the impact of initial community composition (chapter I) and its combination with ocean acidification (chapter II & III) on ecosystem functioning, in this particular case phytoplankton biomass. Overall, the results of this work suggest that predicting ecosystem-level effects of climate change on ecosystem functioning requires shifting away from single-species approaches via multi-species systems towards studies including multiple functional groups.

In order to test the consequences of changing environmental conditions on ecosystem functioning, it is mandatory to understand the role of altered community composition as a major driver of changing ecosystem functioning (Hooper 2012). In nature, priority effects can have major ramifications on community development (Young et al. 2001, Fukami 2005) but have been rarely studied. I could show that priority effects among different species within one functional group affected community composition (chapter I). That is, those species having a numerical advantage at the onset of growth remained dominant during stationary phase. However, despite varying carrying capacities when species were grown in monocultures, these priority effects did not translate into altered community functioning. I consider selective effects and species interactions and subsequent facilitative effects as responsible for the equalization of functioning among the communities. That underlines the fact that single-species experiments are not sufficient to predict whole-community responses. Future studies, investigating the relationship between initial community composition and ecosystem functioning should explicitly take the particular type of species interaction further into account.

When the communities were exposed to elevated CO_2 in the face of global change I found – contrary to my expectation – neither a significant physiological effect nor an ecological effect of elevated CO_2 on biomass (chapter II). I concluded that the lacking effect on ecosystem functioning in this particular model system in response to elevated CO_2 was likely caused by community reorganization due to evolutionary adaptation. The absence of an ecological effect of elevated CO_2, hints towards the possibility that instead we rather found a genotypic selection effect among differentiated strains within species. The additional decomposition of physiological, ecological, and evolutionary effects with respect to community reorganization in response to global change remains a major challenge. A reasonably complete explanation for community-level change in future experiments thus requires considering exploitation of organisms' phenotypic plasticity, shifts in community composition in terms of species or functional groups, and the genetic change within species owing to evolutionary adaptation.

Looking at short-lived organisms with fast generation such as phytoplankton, changes in community composition on the ecological and evolutionary level occur within similar timeframes after the individual species have shifted out of their optima or exhausted their phenotypic plasticity (Ackerly 2003). A conceptual framework to quantify the distribution of all three components is the application of the Price equation (Collins & Gardener 2009). In the present study I used an indirect approach in order to test for evolutionary adaptation. I examined the correlated response to ecological selection (species sorting) and potentially evolutionary genotypic selection as performance of communities selected at elevated CO_2 when exposed back to ambient CO_2 in the species ratio observed in the communities selected at ambient CO_2. However, the most powerful direct test, which was lacking in the present design, would be the comparison of communities selected at elevated CO_2 with those selected at ambient CO_2 both exposed back to elevated CO_2 (Lohbeck et al. 2012). Ideally, one would need to have as additional treatments monocultures exposed vs. not exposed to acidification which would both be used to assemble all possible combinations of communities for an additional decomposition of evolutionary and ecological effects. I suggest comparing the results of a direct experimental decomposition into physiological, ecological, and evolutionary components with those of a Price equation approach for cross-validation.

The studies in chapter I and II both displayed a major shortcoming. Either was based solely on one functional group–coccolithophores. Because phytoplankton functioning depends on trait composition, it remains a major challenge to understand how phytoplankton communities harboring a variety of functional groups will reorganize in response to climate change in order to predict the impact on future oceans' ecosystems (Litchman et al. 2012). It appears that, when looking at more than one functional group, initial community composition can have a much greater impact than elevated CO_2 on phytoplankton biomass, which varied largely among communities (chapter III). I suggest that the different initial ratios between cyanobacteria, diatoms, and dinoflagellates might be the key for the varying competitive and thus functional outcome among the communities. Furthermore, I was able to show that there was an increase in phytoplankton biomass driven by the gain of larger sized diatoms in response to elevated CO_2 which potentially has strong implications for nutrient cycling and carbon export in future oceans. Inherently, using independent natural communities instead of directly manipulating biodiversity, limits the possibility for mechanistic explanation. For future research, I suggest to overcome this limitation by using one known source-community in which biodiversity (i.e. the loss or distribution of given traits) is manipulated in a non-random approach. To date, the majority of research into the functional consequences of changes in community composition is based on biodiversity gradients constructed of randomly assembled communities. Whereas this approach has amplified the knowledge of the mechanisms linking biodiversity and ecosystem functioning (Hooper et al. 2005, Cardinale et

al. 2006), it is limited in its applicability, because the number and relative abundance of species does not change randomly in natural systems (Hutchinson 1959, Menge & Sutherland 1987). Despite this knowledge, the number of studies that explicitly consider the effects of non-random changes in community composition on ecosystem functioning is limited (e.g. Zavaleta and Hulvey 2004, Walker and Thompson 2010). Bracken et al. (2008) highlight the striking differences in the effects of realistic vs. random species-loss scenarios, the latter of which showing no effect. Since, biodiversity is not limited to species diversity, but also encompasses genetic diversity and functional diversity, the latter of which combines different species according to their functional traits, predicting the responses of these different levels of diversity to changing environmental conditions gains center stage when assessing the impacts of global change on ecosystem functioning.

Phytoplankton–the model system used in this work–harbor an extremely diverse, polyphyletic group of microscopic photosynthetic protists (algae) and cyanobacteria that are able to co-occur temporarily and spatially due to trait differentiation in terms of resource requirements and modes of acquisition (Falkowski et al. 2004, Barton 2013, Edwards 2013). Major taxonomic groups of phytoplankton can be classified into distinct functional groups (Iglesias-Rodríguez et al. 2002) with unique biogeochemical signatures. As a consequence, phytoplankton community composition profoundly affects the biogeochemical cycling of many elements, such as carbon, nitrogen, and phosphorous. Due to the wide variety of processes affected, responses to ocean acidification vary broadly across and even within taxa and functional groups. Thus, the changing environmental conditions prevailing on our planet today will likely lead to a shift in community composition, because some species will better cope with the new conditions than others. This leaves the question open of how ecosystem functioning will be affected, since ecosystem functioning depends greatly on the functional characteristics of organisms present in the ecosystem and the distribution and abundance of those organisms over space and time.

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Appendix

Chapter III

Appendix III-A: Final properties of the carbonate system (mean ± SD, n = 4).

Community	1		2		3	
Acidification	Non-acidified	Acidified	Non-acidified	Acidified	Non-acidified	Acidified
TA (µmol kg SW^{-1})	2398 ± 2.6	2405 ± 2.3	2399 ± 1.7	2386 ± 1.4	2399 ± 2.8	2386 ± 2.9
DIC (µmol kg SW^{-1})	1957 ± 7.4	2072 ± 7.2	2008 ± 10.2	2136 ± 12.28	1926 ± 21.83	2073 ± 11.22
pH_T	8.35 ± 0.01	8.19 ± 0.01	8.28 ± 0.01	8.05 ± 0.02	8.39 ± 0.03	8.16 ± 0.02
HCO_3^- (µmol kg SW^{-1})	1646 ± 11	1828 ± 13	1729 ± 16	1941 ± 19	1594 ± 36	1843 ± 18
CO_3^{2-} (µmol kg SW^{-1})	305 ± 4.36	234 ± 6.22	272 ± 6.24	180 ± 7.87	326 ± 14.79	220 ± 7.94
CO_2 (µmol kg SW^{-1})	5.99 ± 0.17	9.64 ± 0.38	7.41 ± 0.31	14.12 ± 0.88	5.28 ± 0.46	10.42 ± 0.58
pCO_2 (µatm)	169 ± 4.69	272 ± 10.6	209 ± 8.71	398 ± 24.71	149 ± 12.98	294 ± 16.37

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Eggers SL, Matthiessen B (2013) Initial dominance in coccolitophore communities affects community structure but does not translate into community functioning. *Marine Ecology Progress Series* 473:67–77.

Matthiessen B, Eggers SL, Krug SA (2012) High nitrate to phosphorus regime attenuates negative effects of rising pCO_2 on total population carbon accumulation. *Biogeosciences* 9:1195–1203.

Eggers SL, Eriksson BK, Matthiessen B (2012). A heat wave and dispersal cause dominance shift and decrease biomass in experimental metacommunities *Oikos* 121:721–733.

Description of the individual scientific contribution to the multiple-author papers

The chapters of this thesis are partly published (chapters I and III) or in preparation (chapter II) for submission to a scientific journal. This list serves as a clarification of contributions to each publication.

Chapter I:

Initial dominance in coccolithophore communities affects community structure but does not translate into altered community functioning

Authors: Sarah Lena Eggers, Birte Matthiessen;

Published in Marine Ecology Progress Series (2013) 473: 67–77

Contributions: SLE and BM conceived research; SLE performed research; SLE analysed the data; SLE and BM discussed the results; SLE wrote the manuscript.

Chapter II:

Physiologically induced shift in the composition of a coccolithophore community does not alter community functioning in response to ocean acidification – but is there a hint towards evolutionary adaptation?

Authors: Sarah Lena Eggers, Aleksandra Magdalena Lewandowska, Birte Matthiessen

In preparation

Contributions: SLE and BM conceived research; SLE and AML performed research; SLE analysed the data; SLE, AML and BM discussed the results; SLE wrote the manuscript.

Chapter III:

Community composition has greater impact on the functioning of marine phytoplankton communities than ocean acidification

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Published in Global Change Biology (in press) doi: 10.1111/gcb.12421

Contributions: SLE, AML, JBR, and BM conceived research; SLE, AML, JBR, SBA, and FG performed research; SLE, AML and BM analysed the data; SLE, AML and BM discussed the results; SLE wrote the manuscript.

Erklärung

Hiermit erkläre ich, dass die vorliegende Dissertation, abgesehen von der Beratung meiner Betreuer, selbstständig von mir angefertigt wurde und dass sie nach Form und Inhalt meine eigene Arbeit ist. Sie wurde keiner anderen Stelle im Rahmen eines Prüfungsverfahrens vorgelegt. Dies ist mein einziges und bisher erstes Promotionsverfahren. Die Promotion soll im Fach *Biological Oceanography* erfolgen. Des Weiteren erkläre ich, dass ich Zuhörer bei der Disputation zulasse.

S L Essen